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DIETARY PROTECTION AGAINST PULMONARY OXYGEN POISONING.(U)
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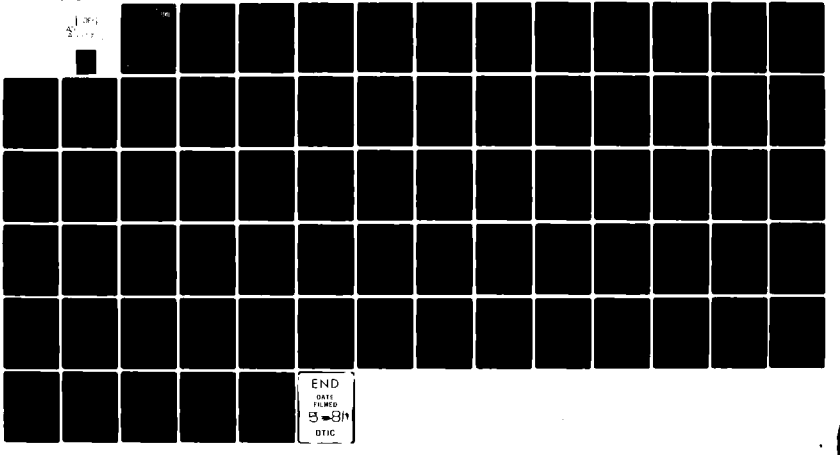

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(6) DIETARY PROTECTION AGAINST PULMONARY OXYGEN POISONING.

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ABSTRACT

This project was designed to determine any influence of dietary composition on susceptibility to pulmonary oxygen toxicity in rats and mice. Of a variety of vitamins given in supranormal doses, only vitamin E proved efficacious in delaying the onset of toxic symptoms in rats exposed to pure oxygen at one atmosphere absolute. Dietary supplementation with the trace element selenium appeared to be beneficial in some experiments but not others. Alteration of amount and type of dietary fat influenced mortality of rats at 1ATA oxygen. The mechanism by which polyunsaturated fats in the diet might change susceptibility to oxygen toxicity was not elucidated, but a possible relationship with pulmonary prostaglandin metabolism is suggested.

Key Words: diet, pulmonary oxygen toxicity, rodents, vitamin E, selenium, polyunsaturated fatty acids, prostaglandins.

INTRODUCTION

The goal of this project was to develop a dietary regimen optimal for attenuation of oxygen poisoning. It was predicated on the fact that cells contain natural antioxidant systems which have evolved to combat overoxidation during normal metabolism in an oxygen-containing environment. Examples include Vitamin E as an integral part of all membranes, the enzyme glutathione peroxidase, some forms of which contain the trace metal selenium, the enzyme superoxide dismutase, the hexose monophosphate pathway of glycolysis which produces reducing equivalents and the sulphydryl-containing substances glutathione as well as sulfur-containing amino acids. The relationship of most known antioxidants is shown in Figure 1.

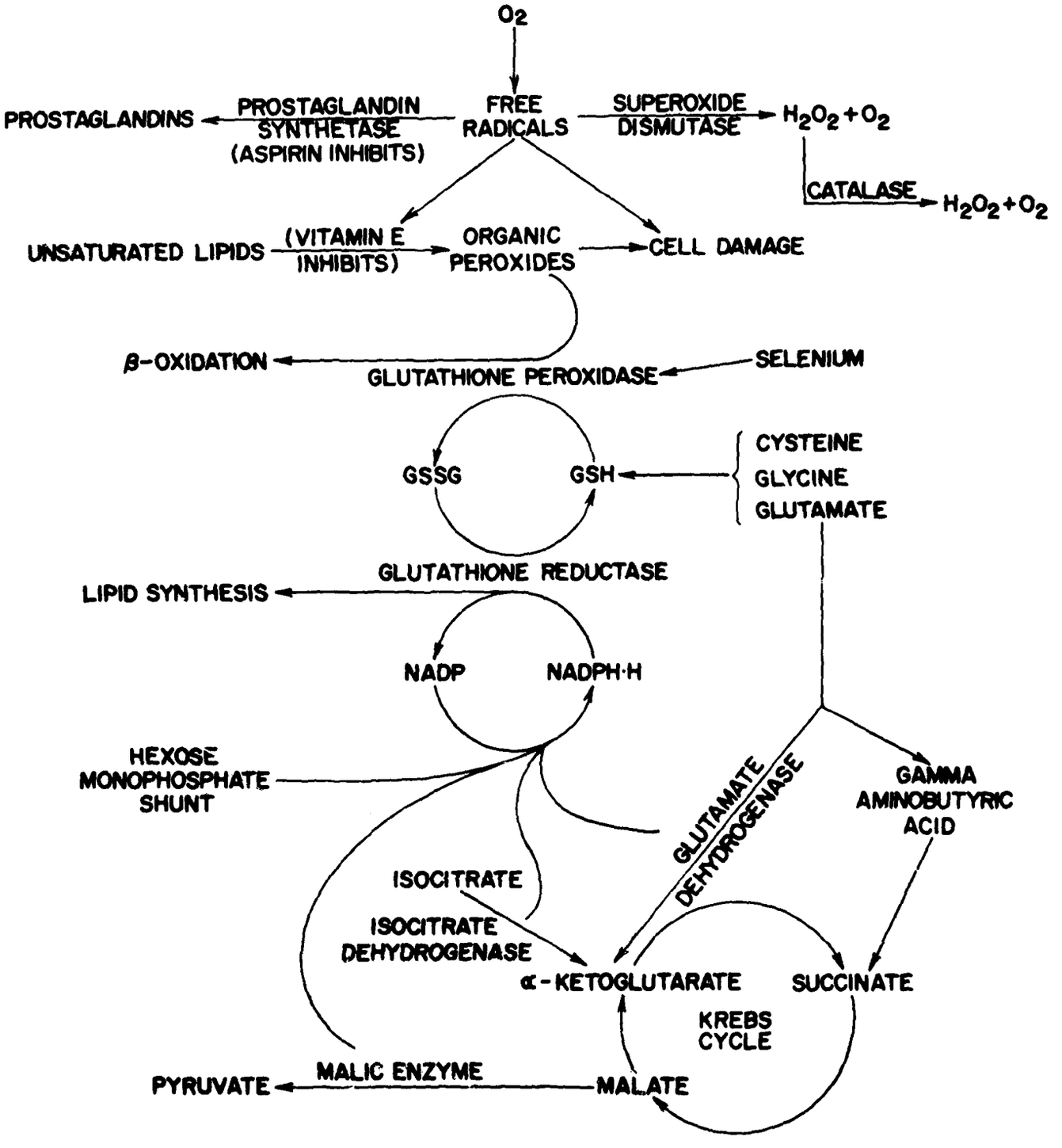
Because these are *naturally-occurring systems*, it was hypothesized that their effectiveness in combatting overoxidation during severe oxidant stress, e.g. hyperoxia, might be amplified by manipulating concentrations of key precursors in the cell or inducing higher enzyme activity. In many cases, the latter can be done by dietary means.

The mechanism of most of the work was to use supranormal dietary concentrations of selected constituents in an attempt to increase cell concentrations, as in the case of Vitamin E, or altering the content of a dietary constituent, such as lipid amount or composition, in order to achieve greater resistance to oxygen toxicity.

The major advantage of using physiological changs in diet is that favorable results allow testing in a range of species and the promise of use in man without untoward side effects or threat to health. In all cases, dietary alterations were restricted to physiologically reasonable changes which could likely be applied with similar qualitative results in man.

This report summarizes chronologically the work performed during this contract by providing a brief introduction to the various experiments followed by the pertinent data, usually in the form of the resulting publication.

Figure 1: Schematic representation of the interrelationships among the known major antioxidant pathways in mammalian cells.



OXYGEN TOXICITY IN MICE

For purposes of screening several dietary constituents, we chose to use the mouse as the experimental subjects. A project in progress at the time of funding of this contract was designed to determine whether or not acclimatization to hypoxia, which had been shown to impart resistance to pulmonary oxygen toxicity at 1 ATA [1], would also do so at higher pressures. The following paper describes the results:

Hall, P., C.L. Schatte and J.W. Fitch. Relative susceptibility of altitude-acclimatized mice to acute oxygen toxicity. J. App. Physiol. 38:279-281, 1975.

We also wished to determine the effects of age and sex on susceptibility to toxicity in both pulmonary and central nervous system toxicity. The following paper relates those results:

Berry, S., J.W. Fitch and C.L. Schatte. Influence of sex and age on the susceptibility of mice to oxygen poisoning. Aviat. Space Environ. Med. 48:37-39, 1977.

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Relative susceptibility of altitude-acclimatized mice to acute oxygen toxicity

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HALL, PETER, CHRISTOPHER L. SCHATTE, AND JOHN W. FITCH. *Relative susceptibility of altitude-acclimatized mice to acute oxygen toxicity.* J. Appl. Physiol. 38(2): 279–281. 1975.—The influence of hypoxic acclimatization at altitudes of 0, 5,000, or 15,000 ft on the relative susceptibility to acute oxygen poisoning was determined in 288 adult female mice. After acclimatization periods of 1, 2, 4, or 8 wk, the mice were exposed to oxygen at high pressures (OHP) of 4, 6, or 9 ATA and the times to convulsion and death recorded. A factorial analysis of variance indicated that altitude and OHP level had inverse, log-linear effects on both parameters. The duration of acclimatization progressively decreased the time to death. The onset of convulsions and death was independent of body weight. There were significant interactions on the measured parameters between various combinations of altitude, OHP level, and duration of acclimatization. While alterations in the metabolism of gamma-aminobutyric acid and high-energy compounds are common to both hypoxia and hyperoxia, the most plausible explanation of the results [illegible] to the decrease in buffer base induced by hypoxic acclimatization which might have caused CO_2 potentiation of OHP symptoms.

hypoxia; acclimatization; oxygen at high pressures; oxygen poisoning; convulsions; death

IN ADDITION to the traditional professions of caisson and underwater work, there is increasing exposure of humans to oxygen at high pressure (OHP) during the treatment of certain diseases, radiotherapy, and sport diving. A substantial number of these people live at an altitude other than sea level and are therefore acclimatized to a reduced inspired oxygen tension.

If hypoxic acclimatization prior to a hyperoxic episode alters the individual's susceptibility to the symptoms of oxygen toxicity, it would be necessary to properly adjust exposure times and maximum OHP levels to maintain safe operating conditions. Brauer et al. (1) have reported that rats acclimatized to an altitude of 17,400 ft (382 mmHg) survived the pulmonary damage attendant upon an OHP level of 1.08 atmospheres absolute (ATA) more than three times longer than sea-level controls. But at 7 ATA, the altitude rats convulsed in half the time of controls.

The present study was undertaken to further describe the relationship between hypoxic acclimatization and the relative susceptibility to convulsions and death resulting from exposure to OHP. Specific emphasis was placed on statistically quantifying the influence of and possible interrelationships between a range of acclimatization altitudes, durations of acclimatization, and OHP levels.

METHODS AND MATERIALS

A group of 300 female mice (CFW strain, Carworth Farms) with a mean ±SD weight of 19.34 ± 1.71 g was raised to adulthood at sea level, randomly divided into three groups and placed in three chambers at altitudes of 0 ft (P_B = 760 mmHg, P_{O_2} = 159 mmHg), 5,000 ft (P_B = 632, P_{O_2} = 135), and 15,000 ft (P_B = 437, P_{O_2} = 89) in air. All were similarly housed and fed (Purina laboratory chow). Once each day, the chambers were briefly opened to ambient pressure (632 mmHg) for servicing. The ranges of chamber temperatures (26–29°C), relative humidities (19–56%), and CO_2 levels (0.06–0.19%) were maintained by adjustment of gas flow through the chambers.

At intervals of 1, 2, 4, and 8 wk, a group of 24 mice was removed from each chamber, weighed, and divided into three groups of eight animals. These groups were exposed to OHP at 4, 6, or 9 ATA (60, 90, or 135 psia) in a hyperbaric chamber (volume = 1,000 liters) fitted with a 24-compartment wood and wirecloth cage which allowed an unobstructed view of all subjects. All oxygen exposures had eight mice from each of the three altitude groups, thereby enabling a valid comparison between groups for every OHP test.

Compression rate was 0.66 ATA/min and a flow was maintained through the chamber such that oxygen analyses of the gas entering and leaving the chamber differed by no more than 0.2% (Servomex paramagnetic analyzer). Chamber temperatures varied between 24 and 26°C during the exposures.

The time to convulsion for each subject was recorded as the time in minutes between reaching pressure and the onset of full clonic spasm. Time to death was taken as the time in minutes between reaching pressure and the last visible respiratory movement.

RESULTS

The mean body weight, time to convulsion, and time to death for each combination of acclimatization altitude, duration at altitude, and OHP exposure is listed in Table 1. The data were analyzed using a factorial analysis of variance for each of the measured parameters; a summary of the statistical results is shown in Table 2. The data for

TABLE 1. *Body weight and times to convulsion and death for each eight-mouse cell as a function of altitude of acclimatization, duration of acclimatization, and oxygen pressure*

Altitude, ft	Duration, wk	O_2 Pressure, ATA	Body Wt, g	Time to Convulsion, min	Time to Death, min
0	1	4	22.1±1.6	135.2±17.9	196.5± 16.6
		6	23.0±1.0	12.0± 4.5	53.8± 6.4
		9	24.0±2.0	3.4± .3	24.1± 2.8
	2	4	24.6±1.5	150.2±39.3	249.1± 82.2
		6	23.7±2.2	8.1± 3.1	43.7± 5.4
		9	23.3±1.8	4.8± 1.9	22.6± 2.1
	4	4	27.8±1.8	157.3±36.4	255.9±100.9
		6	24.7±2.9	18.8±16.6	72.8± 16.1
		9	26.3±2.6	4.1± .6	20.1± 1.3
	8	4	28.6±3.7	179.0±37.4	215.6± 40.6
		6	26.9±2.0	7.7± 2.7	45.9± 4.4
		9	29.0±1.7	3.6± .8	22.2± 2.6
5,000	1	4	21.7±1.5	124.7±31.2	185.3± 22.1
		6	21.5±1.6	7.1± 1.8	48.0± 9.3
		9	21.8±1.2	3.9± .7	23.4± 2.9
	2	4	21.2±2.0	165.8±42.8	277.3± 28.2
		6	21.8±1.3	12.0± 6.3	50.0± 11.9
		9	22.1±2.1	3.1± .3	20.6± 2.7
	4	4	25.0±1.3	157.6±43.3	202.1± 34.4
		6	26.9±2.0	8.7± 4.0	52.4± 4.6
		9	26.1±1.6	3.2± .3	21.0± .5
	8	4	27.6±2.7	176.8±33.8	223.3± 35.3
		6	28.4±1.8	9.2± 6.6	48.3± 11.8
		9	28.4±2.3	3.8± .6	22.0± 1.5
15,000	1	4	21.5±1.1	111.6±25.5	196.7± 14.4
		6	21.0±1.6	4.8± 1.6	45.9± 8.7
		9	21.0±1.6	3.4± 1.5	23.8± 2.6
	2	4	22.6±1.5	54.3±32.1	197.9± 43.0
		6	22.6±1.7	5.4± 3.4	39.7± 11.4
		9	21.9±1.7	3.0± .8	21.9± 1.7
	4	4	22.9±1.6	109.2±44.9	181.7± 62.0
		6	24.5±1.2	4.5± 1.2	46.7± 5.5
		9	23.8±1.2	3.3± .5	20.8± 1.5
	8	4	25.6±1.5	120.9±46.0	169.7± 39.6
		6	26.1±1.6	5.4± 1.9	35.9± 8.3
		9	25.1±1.9	2.9± 1.0	19.8± 1.8

Values are means ± SD.

times to convulsion and death were analyzed using the common logarithmic transformations because a plot of these times versus OHP level revealed an exponential relationship. Further, the means and standard deviations of the 36 eight-mouse groups exhibited a constant ratio for all three OHP levels, a condition for which logarithmic transformation of the data is valid and recommended as a more powerful analysis (8).

Body weight increased significantly throughout the experiment but altitude inversely affected the rate of increase, a finding typical of hypoxic exposure (9). An orthogonal breakdown of the sums of squares indicated that both the effects of altitude and duration at altitudes were linear functions. In addition to these single factor effects, there were significant interactions between the altitude and duration of acclimatization and between altitude and OHP level. However, these interactions were statistically of lesser magnitude than the single factor effects.

By use of correlation and regression tests, body weight was found to have had no effect on any of the other parameters. This agrees with the previously reported findings (1, 5) that oxygen toxicity is independent of body weight within a species.

TABLE 2. *Summary of a factorial analysis of variance for measured parameters*

Parameter	Source of Variation	df	MS	P†
Body wt	Altitude	2	106.54	0.005
	OHP	2	0.56	
	Duration at altitude	3	437.09	0.005
	Alt × OHP	4	11.57	0.025
	Alt × Dur	6	14.35	0.01
	OHP × Dur	6	.92	
	Alt × OHP × Dur	12	5.74	
	Error	252	3.66	
Time to convulsion*	Altitude	2	1.317	0.005
	OHP	2	64.994	0.005
	Duration at altitude	3	0.039	
	Alt × OHP	4	0.333	0.005
	Alt × Dur	6	0.068	0.01
	OHP × Dur	6	0.056	0.025
	Alt × OHP × Dur	12	0.080	0.005
	Error	252	0.023	
Time to death*	Altitude	2	0.124	0.005
	OHP	2	23.575	0.005
	Duration at altitude	3	0.021	0.025
	Alt × OHP	4	0.023	0.005
	Alt × Dur	6	0.020	0.005
	OHP × Dur	6	0.050	0.005
	Alt × OHP × Dur	12	0.009	
	Error	252	0.006	

* Mean square values based on logarithmic transformation of the data. † Probability of Fisher ratio for those factors with $P \leq 0.05$ or better.

The logarithm of time to convulsion varied inversely as a function of altitude and OHP level and the orthogonal analysis indicated that these relationships were primarily log-linear with a small quadratic component. The duration of acclimatization at altitudue had no effect. Statistically significant interactions of lesser magnitude were noted between altitude and OHP level, altitude and duration at altitude, OHP level and duration at altitude, and all three of these factors in combination.

Following a pattern similar to that for time to convulsion, the logarithm of time to death varied inversely as a function of altitude, OHP level, and duration at altitude. A significant variation due to duration at altitude resulted from a drop in time to death at 8 wk; there was no significant fluctuation at 1, 2, or 4 wk time. The relationships of time to death to altitude and OHP level were log-linear with small quadratic components while that for duration at altitude was a combination of linear, quadratic, and cubic functions. The log-linear relationship of time to death is in agreement with previous reports (4) which show an exponential or log-linear pattern for mice over a range of oxygen pressures from 0.2 to 10 ATA.

There were statistically significant interactions of lesser magnitude between altitude and OHP level, altitude and duration at altitude, and OHP level and duration at altitude.

DISCUSSION

The results indicate that acclimatization to hypobaric hypoxia prior to exposure to oxygen at high pressure enhanced the susceptibility of mice to convulsions and death. Predictably, the OHP level was the primary determinant of times to convulsion and death but altitude of acclimatization also had an inverse effect of lesser magnitude on both parameters. Duration of acclimatization at altitude had no effect on time to convulsion but decreased time to death.

Of particular note is the fact that times to convulsion and death showed a log-linear relationship to both altitude and OHP level. This suggests that the influence of either factor might be predicted for a given combination of altitude and OHP exposure.

The results confirm the findings of Brauer et al. (1) that rats acclimatized to 17,400 ft (382 mmHg, Po_2 = 80 mmHg) had a lower mean time to convulsion than sea-level controls during exposure to an OHP level of 7 ATA. The mean decrease in convulsion time for the rats was 50%, which quantitatively agrees with the approximate 37% decrease in our mice maintained at 15,000 ft and exposed to 4, 6, or 9 ATA.

The mechanism by which altitude acclimatization potentiates the effects of OHP cannot be discerned from our data but three possibilities are pertinent. First, the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) has been shown to decrease during exposure to OHP (10) and increase during hypoxia (11), although a simulated altitude of 24,000 ft was required to do so in rats. Since a relatively mild degree of hypoxia was used in the present study and then was detrimental rather than protective during OHP exposure, we do not believe that a common effect of hypoxia and OHP on brain GABA levels provides a reasonable explanation of the results.

The second possible explanation relates to an influence of hypoxia and OHP on brain high-energy compounds, typified by ATP. Sanders et al. (7) have presented evidence suggesting that OHP-induced convulsions result from a decrease in brain ATP concentration below a threshold level. While it is conceivable that hypoxia could reduce brain ATP levels, even acute severe hypoxia (Pa_{O_2} = 22 – 24 mmHg) has been shown to not significantly alter the adenine nucleotide levels in rat brains (6). It therefore does not seem likely that the hypoxic potentiation of oxygen toxicity in this study resulted from a reduction of brain high-energy compounds.

The most plausible explanation of the results relates to an influence of hypoxia and hyperoxia on blood and, presumably, tissue CO_2 content. Hypoxic acclimatization lowers blood CO_2 tension and buffer base (9). Oxygen at high pressure elevates CO_2 tension due to saturation of hemoglobin with oxygen and CO_2 retention concomitant with increased density of the gas mixture (2). Elevation of blood CO_2 potentiates oxygen poisoning while the addition of various buffers affords some protection (2). If the mice acclimatized to altitude did have a reduced buffer capacity, it may be inferred that exposure to OHP elevated blood CO_2 to the point that CO_2 synergism occurred, thereby shortening the times to convulsion and death.

Whatever those mechanisms by which hypoxia influences OHP toxicity may be, our data and that of Brauer et al. (1) suggest that there is an OHP level between 1 and 4 ATA at which hypoxia ceases to protect against oxygen poisoning, but rather enhances it. It may be that oxygen pressure at which the predominant, and ultimately fatal, symptom of oxygen poisoning ceases to be pulmonary deterioration and becomes a central nervous system lesion. Thus, it might be speculated that either a single mechanism of hypoxia exists which protects the lungs but is detrimental to the CNS, or that more than one mechanism is operative and the level of OHP determines which one will predominate.

The results have practical implications in hyperoxic therapy or any other circumstances in which a person acclimatized to hypoxia becomes exposed to oxygen at raised partial pressure. This is particularly important because animal species larger than mice or rats are more susceptible to oxygen poisoning (3) so that they may be affected by altitude to an even greater extent than the mice used in this study. Persons living at altitude who must be treated with hyperbaric oxygen for severe cardiorespiratory dysfunction, radiotherapy of tumors, or infectious diseases may be predisposed to the toxic effects of oxygen. This risk may be further potentiated if other factors which predispose to oxygen toxicity (exercise, hypercapnia, high temperature, certain drugs) are also present. We therefore suggest that physicians or other persons involved with hyperbaric oxygenation adjust exposure times to a given level of OHP on the basis of previous acclimatization to hypoxia.

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Influence of Sex and Age on the Susceptibility of Mice to Oxygen Poisoning

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BERRY, S., J. W. FITCH, and C. L. SCHATTE. *Influence of sex and age on the susceptibility of mice to oxygen poisoning.* Aviat. Space Environ. Med. 48(1):37-39, 1977.

We exposed 360 mice of equal sex distribution to 2, 4, or 6 ATA of pure oxygen at 20, 30, 60, 90, or 120 d of age and recorded the times to death. Female mice survived longer than males at all ages and oxygen pressures tested, an effect more pronounced at 4 and 6 ATA oxygen. All mice showed a consistent decrease in time to death between 20 and 90 d of age; thereafter, the trend was reversed. There were significant interactions between sex and oxygen pressure, and age and oxygen pressure. The mechanisms of these responses could not be discerned, but the data suggested that a post-pubertal increase in the production of gonadal steroids may not have been the determining factor with respect to the sex differences.

THE PROLIFERATION of research on oxygen poisoning has made it clear that its incidence and severity are influenced by a broad range of factors, among which are age and sex. These factors are of obvious interest, both to the investigator seeking mechanisms of oxygen poisoning and the clinician faced with the treatment of young and adult patients of both sexes using hyperbaric oxygenation.

Nevertheless, the reported effects of age, sex, and the administration of gonadal steroids are neither quantitative nor conclusive. Wood (6) noted that female rats tended to survive exposure to 75 p.s.i. oxygen longer than males. Troy and Ford (5) reported that the mean time to convulsion for female rats was significantly longer than that for males when exposed to 75 p.s.i. oxygen. These same authors found that adrenalectomy abolished seizures in all female and some male rats. Subcutaneous administration of estradiol to male adrenalectomized rats doubled their resistance to seizures. On the other hand, guinea pigs of either sex injected with chorionic gonadotropin, progestins, or estrogens, demonstrated a fulminating edema when exposed to 100% oxygen at 1 ATA and died sooner than untreated animals (2).

With respect to age, Hudson and Ferdman (3) observed that newborn mice were substantially more susceptible to pulmonary oxygen poisoning than adults when exposed to 30 p.s.i. oxygen. However, Sperling (4) showed that the severity of lung damage and convulsions induced by exposure to 4.4 ATA oxygen increased with age up to 21 d, after which it plateaued.

This study was undertaken to confirm the sex-related response of mice to acute oxygen poisoning, to describe the post-weaning age-related response, and to ascertain whether or not these factors may be interrelated.

MATERIALS AND METHODS

Two CD-1 mice (Carworth Farm) were transported to and bred at 5,000 ft (632 mm Hg) to produce the subject animals. Over four generations, 360 offspring were raised under uniform conditions of caging, temperature, and lighting. The mice were weaned at 20 d of age, fed Purina Laboratory Chow and water *ad libitum*, and segregated by sex.

At 20, 30, 60, 90, or 120 d of age, 72 animals were randomly selected, weighed and divided into three groups of 24 animals, each with equal sex distribution, for exposure to 100% oxygen at 2, 4, or 6 ATA. Because no single generation of mice produced a number sufficient to test all five age groups simultaneously, each generation was allowed to mature to a selected age: first generation, 90 d; second generation, 60 d; third generation, 120 d; fourth generation, 20 and 30 d. The pressures were chosen with the anticipation of differentiating any pressure-related effects of age or sex. Exposure to 100% oxygen resulted primarily in pulmonary symptoms at 2 ATA, primarily in CNS seizures at 6 ATA, and both with about equal incidence at 4 ATA. All exposures took place in a 1,000-l chamber fitted with a 24-compartment wood and wire-cloth cage, which allowed observation of individual animals. Compression rate was 0.66 ATA/min and gas flow through the chamber was maintained at a rate sufficient to keep $\%O_2 < 0.5\%$. Chamber temperatures ranged from 21-24 C. All exposures began between 0800 and 1000 hours, and ended when all animals were dead. Time to death was taken as the time in minutes between reaching pressure and the last visible respiratory movement (error = ± 45 s at 4 and 6 ATA and ± 4 min at 2 ATA). The data were analyzed factorially for variance. Fisher probability values of 5% or less were considered significant.

TABLE I. MEAN ± S.D. TIMES TO DEATH, IN MINUTES, OF MICE AS A FUNCTION OF AGE IN DAYS, SEX, AND OXYGEN PRESSURE IN ATMOSPHERES ABSOLUTE. EACH MEAN REPRESENTS 12 OBSERVATIONS.

Age	Pressure	Minutes	
		Female	Male
20	2	1954 ± 169	1707 ± 111
	4	339 ± 31	262 ± 60
	6	111 ± 11	77 ± 25
30	2	1735 ± 72	1521 ± 80
	4	318 ± 65	251 ± 56
	6	83 ± 27	58 ± 6
60	2	1560 ± 133	1284 ± 229
	4	277 ± 64	219 ± 47
	6	78 ± 24	54 ± 14
90	2	1391 ± 86	1250 ± 136
	4	233 ± 53	184 ± 18
	6	51 ± 8	45 ± 7
120	2	1482 ± 106	1371 ± 121
	4	307 ± 55	188 ± 47
	6	61 ± 11	43 ± 8

RESULTS AND DISCUSSION

The mean times to death of the various groups are listed in Table I. There was a consistent decrease in time to death of both sexes between 20 and 90 d of age at all three oxygen pressures. Between 90 and 120 d of age, there was a reversal of this trend. Female animals had consistently longer times to death than males, the differences becoming greater with increasing oxygen pressure. The relative percentage increase in mean time to death of females over males was 14, 34, and 37% at oxygen pressures of 2, 4, and 6 ATA, respectively.

A summary of a factorial analysis of variance of times to death is shown in Table II. The effects of both sex and age were highly significant as were interactions between sex and pressure, and age and pressure. There was no effect of body weight on time to death.

Our results were not designed to provide information concerning the mechanisms by which sex and age might alter the response to oxygen poisoning. However, one feature concerning the possible role of the sex steroids may be pertinent. Mice attain reproductive capacity between 28 and 50 d of age (1), at which time it would be expected that the production of estrogens by females and androgens by males would be considerably higher than that prior to puberty and fertility. If this were the case, any alteration in the susceptibility to oxygen poisoning effected by changes in the levels of the gonadal steroids would be expected to have been manifested at 60 d and beyond. Such a trend was not apparent. As indicated in Table II, there was no interaction between age and sex, i.e., the effect of sex did not change with age. This is graphically depicted in Fig. 1, in which it may be observed that the difference in the mean time to death at all oxygen pressures between males and females remained relatively constant from 20 to 120 d of age.

TABLE II. FACTORIAL ANALYSIS OF VARIANCE OF TIMES TO DEATH.

Source	df	F	P
Sex	1	125.38	<0.00001
Age	4	76.36	<0.00001
Pressure	2	11032.62	<0.00001
Sex × age	4	1.47	0.21021
Sex × pressure	2	35.98	<0.00001
Age × pressure	8	37.54	<0.00001
Sex × age × pressure	8	1.79	0.07693
Error	330		
Total	359		

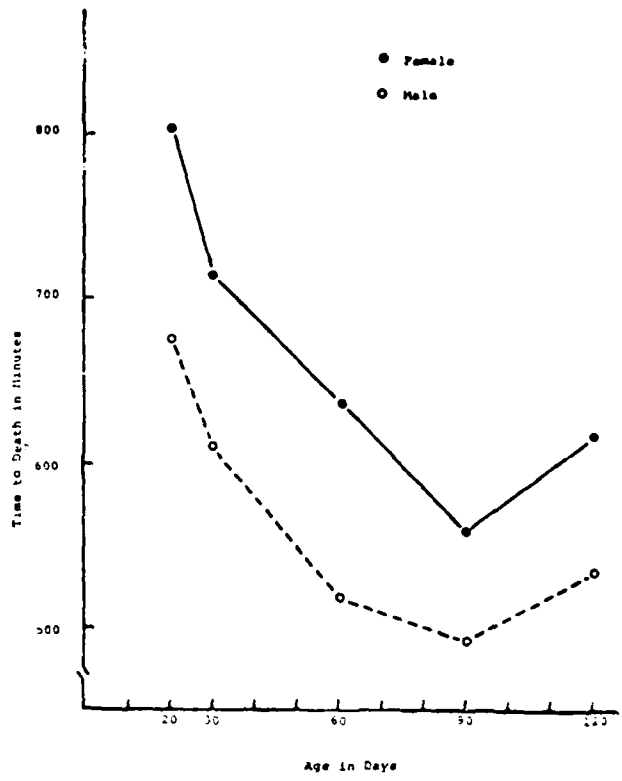

Fig. 1. Grand mean times to death in minutes for all oxygen pressures of male and female mice as a function of age in days.

The reason for the increase in death times of both male and female mice between 90 and 120 d of age is unknown. Because this trend was observed in both sexes and at all three oxygen pressures, we believe it probably represents a legitimate age-related effect rather than an artifact.

With regard to the effect of age on the response to oxygen poisoning, we have extended the work of Sperling (4) and found that increasing age is associated with a decrease in tolerance to hyperbaric oxygen up to at least 90 d of age. To the researcher investigating mechanisms of oxygen poisoning, the inverse relationship between tolerance to oxygen poisoning and age suggests biochemical aspects of CNS maturation as possible determinant factors. To the clinician treating patients ranging from infants to adults, this relationship suggests that age may be one of several variables which should be considered when determining optimum oxygen exposure regimens. Our results, and those of Sperling

(4), further indicate that the age-related response to hyperoxia can be sufficiently defined to allow the prediction of an individual's relative susceptibility to a given oxygen exposure intensity and duration based on age as well as sex.

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Nerve division for ischemic foot pain

Peripheral nerve division can provide pain relief in selected patients with nonreconstructible arterial occlusion, ischemic forefoot lesions, and unbearable foot pain.

Under most circumstances, limbs of patients with favorable segmental lesions at any level in the extremity should be revascularized and if arterial reconstruction is impossible, amputation is the operation of choice and not necessarily an admission of failure. Indications for nerve division, however, include severe and chronic pain, local gangrene, nonreconstructible arterial lesions, absence of overt infection, predicted inability to heal a lesser amputation, and refusal of major amputation.

Contraindications are active sepsis, pain localized to the heel, and predominance of neuropathic pain.

Although the operation produces anesthesia of the forefoot and paralysis of the intrinsic foot muscles, proprioception is not lost, and ambulation offers no difficulty. Local ischemic lesions remain dry and contained with conservative foot care. Patients with both diabetic neuropathy and ischemia have delayed symptomatic relief that is not as complete as that of patients without neuropathy.

Scrupulous hemostasis must be maintained during surgery, since the vasa vasorum or nervosum may be torn during nerve immobilization. The length of the nerve removed between clips varies with the exposure and does not have an observed effect on the adequacy or extent of forefoot anesthesia. Delayed wound healing may be a problem despite gentle tissue handling and adequate hemostasis.

During a three-year interval, 12 multisensory peripheral nerve divisions were performed in 10 patients. Only two had to have subsequent limb amputation while seven patients have been ambulatory with minimal foot pain for three months to two and one-half years.

JOSEPH ALPERT, MD, DONALD K. BRIEF, MD, BRUCE J. BRENER, MD, and VICTOR PARSONNET, MD, Newark Beth Israel Medical Center and College of Medicine and Dentistry of New Jersey, Newark. Peripheral nerve division for relentless ischemic foot pain. *Arch Surg 111:557-560, 1976.*

DIETARY ANTIOXIDANT SUPPLEMENTATION

The initial experiments were designed to screen dietary compounds likely to affect natural cellular antioxidant systems. In almost all experiments performed under this contract, a semi-purified diet having a content similar to that of the average American was used as the basal diet. Desired changes could then be made for trace constituents without changing diet composition or consistency.

Results of supplementation with vitamins E and K, selenium and the sulfur amino acids are described in the following paper:

Schatte, C. and A. Swansinger. Effect of dietary anti-oxidant supplementation on the susceptibility to oxygen toxicity in mice. Aviat. Space Environ. Med. 47:147-150, 1976.

Additional experiments supplementing vitamins A, C and several congeners of K were without effect.

The parenteral administration of vitamin E and Se to mice prior to oxygen exposure showed that high blood and tissue levels of E protected against oxygen poisoning while Se did not (Figure 2). It also indicated that dietary vitamin E probably did not get to the tissues in high amounts and that higher levels than those used in the feeding experiments were needed to observe an effect on oxygen poisoning.

Effect of Dietary "Antioxidant" Supplementation on the Susceptibility to Oxygen Toxicity in Mice

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SCHATTE, C., and A. SWANSINGER. *Effect of dietary "antioxidant" supplementation on the susceptibility to oxygen toxicity in mice.* Aviat. Space Environ. Med. 47(2):147-150, 1976.

This study was undertaken to test chronic feeding of some normal dietary constituents on susceptibility to oxygen toxicity. Eight-week-old male CD-1 mice were fed a semi-purified diet simulating that of the average American male and supplemented with either vitamin E, vitamin K, selenium, or the sulfur amino acids methionine and cystine. After 2, 4, 8, or 16 weeks, groups of mice were exposed to oxygen at 1, 4, or 8 ATA and times to respiratory distress, convulsion, and death recorded. Vitamin E and amino acid supplementation had no effect whereas vitamin K and selenium supplements increased time to death at 1 ATA. Only the effect of selenium was statistically significant. All diets significantly increased the time of onset of the measured parameters beginning after 4 weeks, suggesting that one or more constituents of the basal diet afforded some protection against oxygen toxicity.

THE INCREASING incidence of exposure to oxygen at high pressure during diving and medical treatment has spurred interest in the mechanisms of oxygen toxicity and possible prophylactic therapy. Several agents have shown promise in attenuating the effects of oxygen poisoning although many appear to have undesirable side-effects which may restrict their use (4,6,12,16,19). Among these agents are some normal dietary constituents which, when administered acutely, can prolong the onset of oxygen poisoning and which do not appear to have toxic side effects when administered at high dosages (9,11,13,15,22).

The fact that certain normal dietary constituents can influence the susceptibility to oxygen poisoning suggests that an organism's diet might be formulated so as to enhance protection against the symptoms of oxygen toxicity. If increased cellular incorporation of these substances, as a result of chronic dietary supplementation, were to decrease susceptibility to the pulmonary and central nervous system manifestations of oxygen poisoning, persons might be safely treated by means of diet prior to a hyperoxic episode. Toward this end, a study was designed to investigate on a preliminary basis four so-called antioxidants (vitamins E and K, selenium, and the sulfur amino acids methionine and cystine), the incorporation of which would be expected to increase during chronic supplementation, which are not toxic in physiological doses, and which might be prophylactic to the symptoms of oxygen poisoning.

MATERIALS AND METHODS

Eight-week-old male CD-1 mice (Charles River Laboratories) were used in two experiments. The mice were housed and fed under the same conditions in both studies. The basal diet used was designed to approximate that of the average American male (3) and contained by weight 19% protein, 21% fat (saturated/unsaturated ratio of 3:2), and 51% carbohydrate. The composition is listed in Table I.

In the first experiment, one group of mice was given supplemental selenium as Na_2SeO_3 (10 ppm vs. 0.1 ppm for controls) and another α-tocopherylacetate (600 i.u./kg. diet vs. 60 i.u./kg. diet for controls). In the second experiment, the two experimental groups were given vitamin K_3 (menadione bisulfite, 100 mg/kg diet vs. 1 mg/kg diet for controls) or the sulfur amino

TABLE I. COMPOSITION OF BASAL DIET. ALL CONSTITUENTS LISTED IN g/kg DIET.

Lactalbumin	190
Cornstarch	253
Sucrose	250
Beef Tallow	127
Safflower Oil	85
Cellulose	25
Williams—Briggs salt mix[a]	50
Vitamin mix[b]	20

[a]Supplemented with Na_2SeO_3 to give 0.1 ppm Se/kg diet.
[b]Contains per 2 kg of mix made up with cornstarch: vitamin A acetate, 400,000 IU; D_3 40,000 IU; dl-α-tocopherylacetate, 5,500 IU; menadione bisulfite, 100 mg; biotin, 50 mg; B_{12} in mannitol X 1000 10 g; Ca pantothenate, 2 g; choline Cl, 400 g; folate, 200 mg; PABA, 20 g; inositol, 20 g; niacin, 3 g; pyridoxine HCl, 500 mg; riboflavin, 500 mg; thiamin, 500 mg; ascorbic acid, 10 g.

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acids methionine and cystine (each at 0.6% vs. ~ 0.3% for controls). These levels of supplementation were selected as being sufficiently high to be effective yet physiological.

At intervals of 2, 4, 8, or 16 weeks after beginning the diets, 24 mice were randomly picked from each dietary group, divided into three groups of eight each, and exposed to 100% oxygen at 1, 4, or 8 ATA. These pressures were selected with the intention of demonstrating any selective influence of the dietary supplements. It was anticipated that supplements influencing primarily the pulmonary symptoms might be most effective at 1 ATA and those having a more central effect might protect against symptoms at 4 and 8 ATA. If a single mechanism of oxygen toxicity exists, it was anticipated that prophylactic supplements would be effective at all three oxygen levels.

The exposures at 4 and 8 ATA were carried out in a 1000-*l* chamber containing a wood and wirecloth cage with 24 separate compartments; all subjects could therefore be observed individually. The 1 ATA exposures were made in a thermostated 300-*l* chamber. The mice were allowed to run free and had food and water *ad lib*. A 12:12-h light cycle was maintained during the 1 ATA exposures.

All exposures were carried out with eight mice from each of the two experimental groups and eight controls. Compression was at a rate of 1 ATA/min, carbon dioxide was maintained at $<0.5\%$ as measured by gas chromatography, and temperature was held at 25-26°C.

At 4 and 8 ATA, the times to respiratory distress (gasping), clonic convulsion, and death were recorded. The chamber was checked at 4-h intervals during the 1 ATA exposures and time to death recorded with an accuracy of ± 2 h.

The data were analyzed for variance using a 2×3×4 design for times to respiratory distress and convulsion and a 3×3×4 design for time to death. Statement of statistical significance refers to a Fisher ratio of $p<0.05$ or better.

RESULTS

In the first experiment, there was no significant effect of diet or duration of feeding on any parameter measured during exposure to 4 or 8 ATA. There was, however, a significant effect of diet and duration of diet feeding on time to death of mice exposed to OHP at 1 ATA (Fig. 1). Se-supplemented mice had significantly prolonged times to death when compared to controls. Although not statistically significant, animals fed α-tocopherylacetate showed consistently shorter times to death than controls.

Fig. 1 shows that the influence of duration of feeding manifest itself primarily as a substantial increase in time to death after 4 weeks. While the duration of diet feeding did not significantly alter parameters measured during the 4 and 8 ATA exposures, times to respiratory distress, convulsion, and death exhibited a similar pattern as time to death at 1 ATA, i.e., a substantial increase in the time to onset of these symptoms at 4 weeks.

In the second experiment, identical to the first except

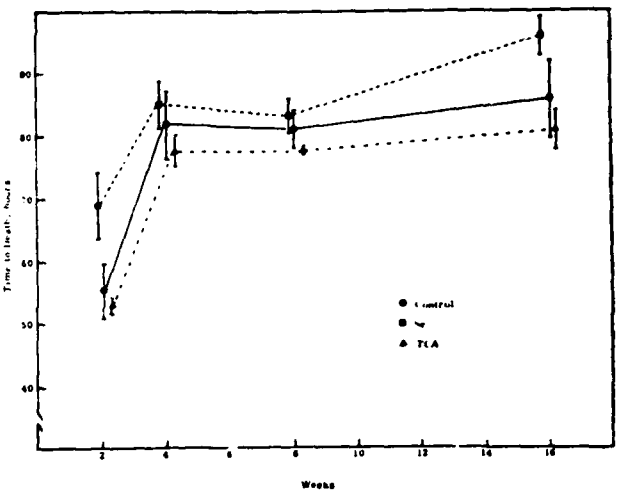

Fig. 1. Mean ± S.E.M. time to death of mice supplemented with selenium (Se) or α-tocopherylacetate (TCA) during exposure to 100% oxygen at 1 ATA. (n=8).

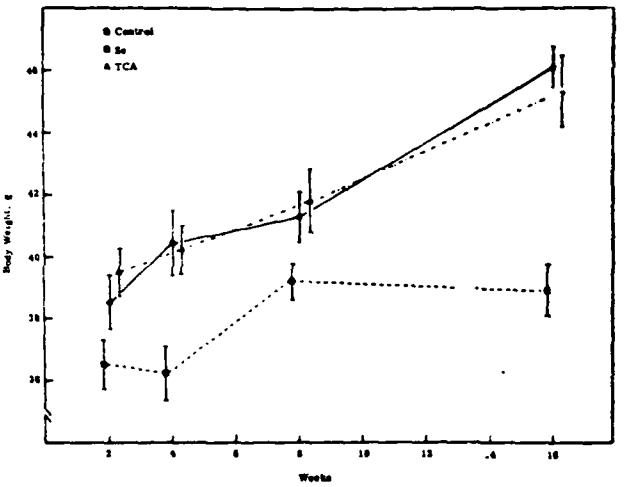

Fig. 2. Mean ± S.E.M. body weight of mice supplemented with selenium (Se) or α-tocopherylacetate (TCA) for 2, 4, 8, or 16 weeks. (n=24).

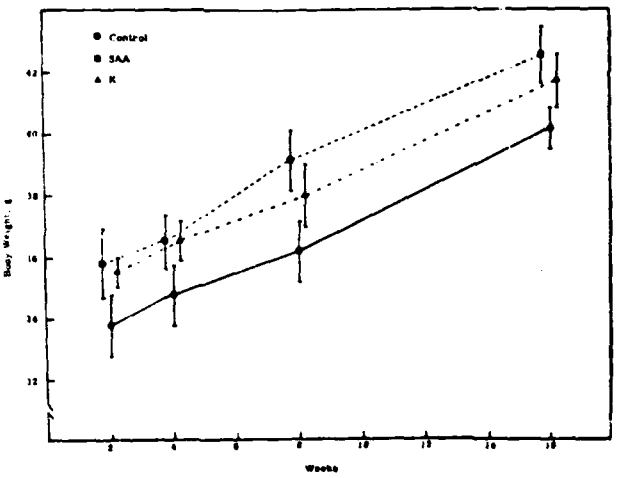

Fig. 3. Mean ± S.E.M. body weight of mice supplemented with vitamin K_3 (K) or sulfur amino acids (SAA) for 2, 4, 8, or 16 weeks. (n=24).

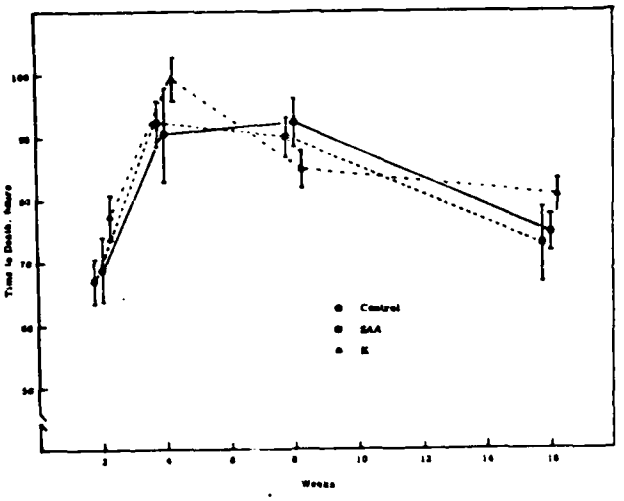

Fig. 4. Mean ± S.E.M. time to death of mice supplemented with vitamin K_3 (K) or sulfur amino acids (SAA) during exposure to 100% oxygen at 1 ATA. (n=8).

testing the effects of the sulfur amino acids methionine and cystine, there was no significant effect of diet on any parameter except body weight (Fig. 3). Both experimental groups gained weight at a faster rate than did controls. It may be seen in Fig. 4, however, that animals supplemented with vitamin K_3 had longer times to death during exposure to oxygen at 1 ATA than controls after 2, 4, and 16 weeks; the unexplained decrease in time to death at 8 weeks precluded a statistical difference between the K-treated mice and controls.

There was a significant effect of duration of diet feeding on times to respiratory distress at 4 ATA, time to convulsion at 4 and 8 ATA, and time to death at 1, 4, and 8 ATA. The pattern that duration of feeding had is represented in Fig. 4. As in the case of the first experiment, there was a substantial increase in the parameters at 4 and 8 weeks when compared to 2 weeks. Peculiar to the second experiment, there was a large decrease in all values at 16 weeks, the cause of which is uncertain.

Analyses of brain and lung for selenium, total tocopherol, and total sulfhydryl groups are being completed and are not reported here. Based on the data available thus far, there appears to be a general increase with duration of feeding in the tissues levels of supplemented substances, the increase being more pronounced in brain. That selenium did accumulate in the Se-treated mice is suggested by the depressed rate of weight gain in these animals (Fig. 2), a finding associated with marginal Se toxicity (8).

No analyses for vitamin K concentration were attempted.

DISCUSSION

The principal findings of this study are: 1) supplemental selenium can significantly increase time to death in mice exposed to 100% oxygen at 1 ATA, 2) vitamin K_3 gives some indication of a similar effect, 3) under the conditions of these experiments, supplemental α-tocopherylacetate or sulfur amino acids do not provide protection against oxygen poisoning, and 4) one or more components of the basal diet, other than the experimental variables, substantially increased survival times after being fed for 4 weeks.

Selenium: Selenium has an apparent "antioxidant" function in the cell which is related to vitamin E function (5). It has a known but not necessarily exclusive role as a component of glutathione peroxidase (GSH-Px) which is thought to catalyze the destruction of lipid peroxides by using them to oxidize glutathione (10). Levels of GSH-Px have been shown to be directly dependent on Se levels in the diet (2). It may be speculated that, if Se-treated mice in this study had elevated GSH-Px levels, the prolongation of time to death may have been a result of an increased ability to cope with lipid peroxides formed during oxygen exposure. Because selenium enhances the effectiveness of the sulfur amino acids at least *in vitro* (1), a more efficacious route of administration might be as seleno-amino acids rather than as selenite.

α-Tocopherylacetate (TCA): Vitamin E is a well-known "antioxidant" which has been shown by several studies to attenuate the onset and severity of oxygen toxicity (13,15,20,22). But in most cases, supplemented animals were compared with deficient controls such that rather dramatic differences were obtained. In one study employing acute supplementation of TCA above dietary requirements, TCA had no effect on times to convulsion or death in mice or lung damage in rats (12). However, chow-fed mice injected acutely with TCA have been shown by Mengel's group to be protected against hemolysis and seizures resulting from exposure to 100% oxgyen at 45 psia (13,22).

In those studies, the vitamin was administered in higher doses and over a shorter period of time than in the present work. It is possible that those animals had relatively high blood levels but relatively low cellular incorporation. It has been demonstrated that at least one agent prophylactic to oxygen poisoning, lithium, is effective at peak blood levels but not at peak tissue levels (16). If such is the case for TCA, it might explain the discrepancy between Mengel's results and ours. Whatever the explanation, our results indicate that chronic supplementation of TCA at moderate doses does not enhance protection against the symptoms of oxygen poisoning despite apparent increases in brain and lung tissue concentrations.

Methionine and Cystine: These amino acids have been shown to have an "E-sparing" effect (17), perhaps via their formation into reduced glutathione which is known to protect against oxygen toxicity (4,19,21). Supplementation of these amino acids in the diets of chicks can increase tissue glutathione levels (14) and the preliminary indications are that both protein and non-protein sulfhydryl groups were increased in the brains and lungs of treated mice in this study. If the increased non-protein sulfhydryl concentration reflected true glutathione concentration, the lack of any protective effect is intuitively puzzling. However, it should be noted that previous reports concerning the prophylactic effect

of glutathione involved acute administration of the compound (4,19,21) such that blood, but perhaps not tissue, levels were elevated. If this was true, it may be speculated that glutathione, like lithium and perhaps vitamin E, may be more effective extracellularly than intracellularly. An alternative possibility is that the rate of cellular glutathione synthesis is relatively slow such that endogenous pools are quickly consumed during oxygen exposure; an exogenous source administered prior to or during oxygen exposure might prevent severe depletion temporarily, thereby prolonging the breakdown of the system. Further, the ability of the glutathione system to cope with cellular over-oxidation may be limited by glutathione utilization rather than total amount of glutathione such that quantitative increases of cellular glutathione may not be too important (18).

Vitamin K: Like selenium and the sulfur amino acids, vitamin K can replace vitamin E to some extent, suggesting that it has some antioxidant activity (17). Supplementation of both the water-soluble (K_3) and oil-soluble (K_1) forms has been reported to increase survival of mice following exposure to oxygen or irradiation (11).

Our results suggest that chronic supplementation with moderate doses of K_1 has some protective effect against the pulmonary symptoms of oxygen poisoning at 1 ATA. The mechanism of action is unclear, although it probably acts in its postulated role as a redox cofactor in the electron transport system (7). Vitamin K has several analogues with varying physicochemical properties; it is possible that the most efficacious form has yet to be tested.

Effects of Duration of Feeding: Perhaps the most intriguing observation of these two experiments was the marked (20-50%) increase in death times at 1 ATA which occurred after 4 weeks' feeding. Similar but less pronounced patterns were observed in the second experiment at 4 and 8 ATA. Since all three diets effected the same change, it is tempting to speculate that one or more components of the basal diet, other than the experimental variables, enhanced a critical reaction between 2 and 4 weeks feeding duration such that the onset of oxygen poisoning was prolonged.

It cannot be ascertained from the present data what these components might be, but we have not observed such an increase in other experiments in which standard Laboratory Chow was fed. The most dramatic difference between the synthetic diets used here and Laboratory Chow is the fat content (21% vs. ~ 4%, respectively). We have previously shown (unpublished results) that the fatty acid content can influence susceptibility to oxygen convulsions and we are, therefore, pursuing the possibility that the fat content of the average American diet may influence susceptibility to oxygen poisoning.

Although normal dietary constituents may not prove to be as efficacious as some synthetic compounds which may eventually come into use, it is clear that diet can play a role in attenuating the onset and severity of oxygen poisoning.

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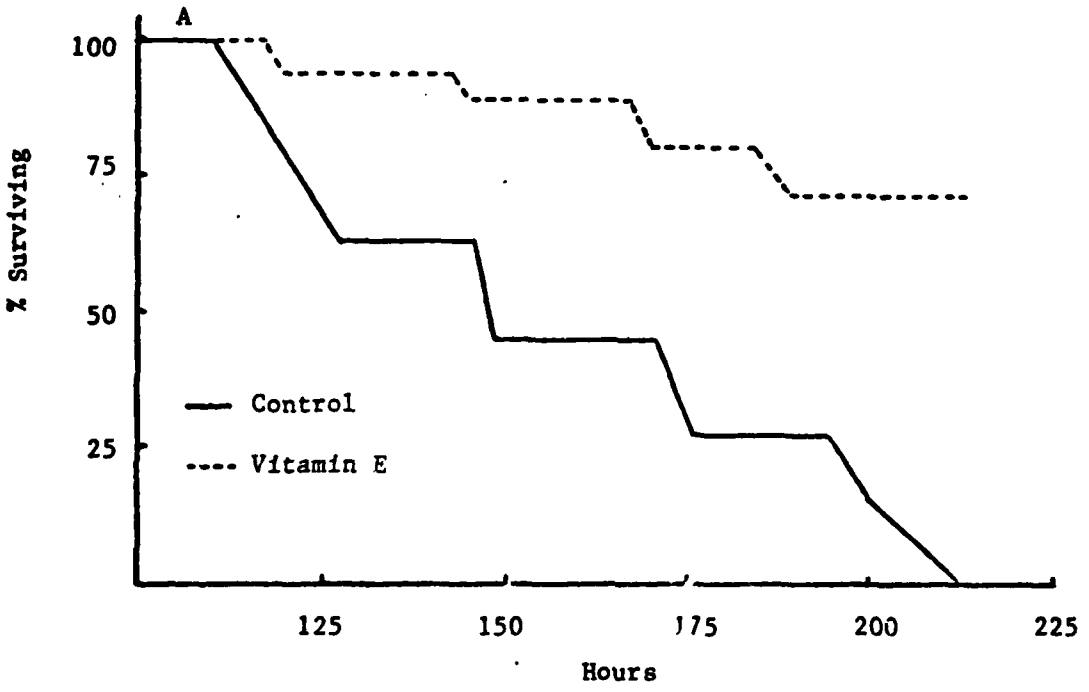
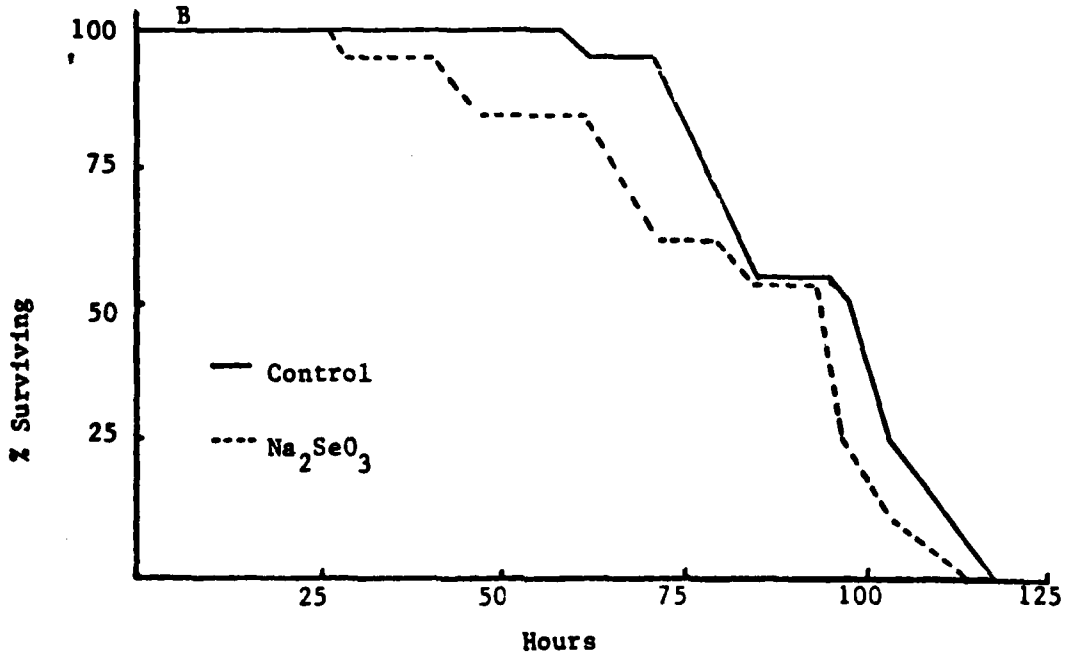
Figure 2. Survival of mice injected intraperitoneally with vitamin E in peanut oil (A) or $Na_2 SeO_3$ in saline (B) during exposure to 1 ATA oxygen. Each group contained 12 mice.





PULMONARY ANTIOXIDANT ENZYMES

We wished to evaluate vitamin E and selenium on pulmonary parameters prior to mortality. It had been demonstrated that the activity of certain antioxidant enzymes, e.g. glutathione peroxidanse, the hexose monophophate shunt and superoxide dismutase, increased during oxygen exposure and thus might be used as an index of oxidant stress[2]. We therefore evaluated changes in the activities of these enzymes as a function of treatments thought to protect against oxygen poisoning--pre-treatment with hypoxia, sex and dietary supplementation with vitamin E and selenium.

We switched to the rat as an experimental subject because of its larger size for tissue sampling and better-defined dietary requirements.

Table 1 shows that the antioxidant enzymes are not different in male vs. female rat lungs prior to or after 48 hours of hyperoxia.

Table 1. Mean ± SD Lung Weights (g) and Enzyme Activities (e.u./100 mg Fresh Tissue) in Rats (N = 4).

Sex	Hours Exposure	Lung Weights	GSH-Px	HMP	SOD
Male	0	1.53 ± .17	.62 ± .04	.23 ± .02	1.22 ± .11
	24	1.66 ± .05	.73 ± .07	.26 ± .02	.87 ± .14
	48	1.96 ± .27	.51 ± .07	.09 ± .03	.61 ± .16
Female	0	1.28 ± .10	.68 ± .05	.18 ± .02	1. _ .38
	24	1.81 ± .63	.70 ± .06	.26 ± .08	.89 ± .25
	48	1.99 ± .29	.52 ± .03	.07 ± .02	.66 ± .19

Table 2 shows that rats acclimatized to hypoxia prior to oxygen exposure do not have different activities of the antioxidant enzymes.

Table 2: Mean ± SD Lung Weights (g) and Enzyme activities (e.u./100 mg Fresh Tissue) for Normoxic (N) and Hypoxic (H) Rats Following 0, 24 or 48 Hours of Oxygen Exposure (n = 4).

Treatment	Lung Weight	GSH-Px	HMP	SOD
N-0	1.40 ± .17	0.51 ± .03	0.31 ± .07	0.67 ± .12
N-24	1.49 ± .13	1.18 ± .12	0.28 ± .01	0.61 ± .17
N-48	1.82 ± .44	0.43 ± .10	0.32 ± .10	0.54 ± .10
H-0	1.98 ± .11	0.51 .08	0.29 ± .01	0.58 ± .06
H-24	1.81 ± .20	1.01 ± .07	0.31 ± .03	0.61 ± .08
H-48	1.59 ± .31	0.51 ± .05	0.28 ± .06	0.56 ± .05

An attempt to induce elevated hexose monophosphate activity in lungs by the use of a low fat, fasting-refeeding technique was successful in liver but not in lung (Table 3).

Table 3: Mean ± SD Enzyme Activities Expressed as Units per 100 mg Fresh Tissue (n = 5).

Enzyme		Fat Content			
	Tissue	0%	1.2%	1.2%(F)	21.2%
HMP	Liver	2.37 ± 1.28	1.14 ± .26	2.92 ± 1.92	0.31 ± .14
	Lungs	0.32 ± .08	0.36 ± .04	0.37 ± .03	0.32 ± .07

Dietary selenium and vitamin E as a possible prophylactic to pulmonary oxygen poisoning

C. L. Schatte

INTRODUCTION

The scope of oxygen therapy is limited largely by its potential toxicity. If the toxicity could be ameliorated, the therapeutic use of oxygen might be extended. With the goal of delaying the onset of pulmonary oxygen poisoning during continuous exposure at 1 ATA, I have experimented with the dietary manipulation of the natural antioxidant systems of pulmonary cells prior to oxygen exposure. This approach has the advantage of enhancing the cells' defence mechanisms by using normally consumed substances. The extensive literature on the pharmacological effects of many of these substances in humans implies that their potential clinical use may be facilitated.

The cells' antioxidant defences apparently can detoxify metabolically produced oxidants at their normal rate of production, which is pO_2 dependent, (superoxide and peroxide radicals) but they cannot cope when the pO_2 is increased.

The 'core' of the cells' antioxidant defence is probably the vitamin E—glutathione peroxidase system. Vitamin E (E) probably acts to preferentially prevent peroxidation of membrane polyunsaturated fatty acids. Lipid peroxides which do form are detoxified by a series of reactions, the most important of which is catalysed by glutathione peroxidase (GSH-P_x).

Several factors in the GSH-P_x system are diet-dependent. One form of GSH-P_x requires selenium (Se), the level of which in the diet regulates the enzyme's activity.[1] Thus, a key antioxidant system enzyme can be induced to supra-normal activity by elevating dietary Se. Glutathione (GSH) synthesis requires cysteine, glycine and glutamate, the latter also serving as a source of reduced phosphopyridine nucleotide (NADPH H) formation and as a precursor of the neurotransmitter gamma-aminobutyric acid which has been implicated in the aetiology of oxygen-induced convulsions.[2,3]

The activity of hexose monophosphate shunt (HMP) enzymes which help control the rate of NADPH H production—involved in the complex GSH-P_x system—can be increased in liver by diet.[4,5]

The importance of E-GSH-P_x as an antioxidant system is illustrated by the fact that GSH, GSH-P_x HMP enzymes and GSH reductase have all been shown to increase during adaptation to hyperoxia.[6]

Proc. 6th int. cong. on HYPERBARIC MEDICINE, 1977, pp. 84-91. Aberdeen University Press

METHODS AND MATERIALS

The experiments were designed to determine prophylactic effects of dietary supplementation with E and Se alone and in combination, on the onset of pulmonary oxygen toxicity. Weanling male Sprague-Dawley CD-1 rats (Charles River Company) were fed a semi-purified diet (Table 1) similar to that of the average American[7] supplemented with nothing, 6000 ppm E (100 × control) 1 ppm Se (30 × control) or both. After feeding periods of 4, 8 or 12 weeks, the rats were exposed to pure oxygen at 1 ATA for 24 or 48 hours. At the 4-week testing, the oxygen exposures were 36 and 60 hours but unexpectedly high mortality at

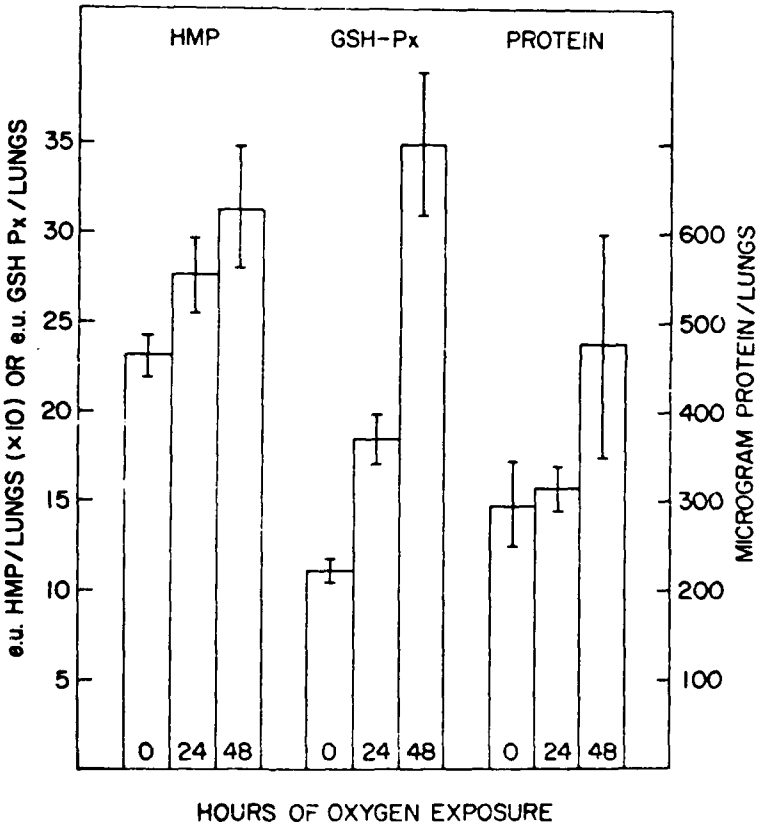


Fig. 1 Response of lung glutathione peroxidase ($GSH-P_x$), hexose monophosphate shunt enzymes (HMP) and lung wash protein content (Protein) in rats exposed to pure oxygen at 1 ATA for 0, 24 or 48 hours.

60 hours (see Results) necessitated the 24- and 48-hour exposures at 8 and 12 weeks.

After oxygen exposure the lungs were removed and lavaged. Lung washes were analysed for protein content,[8] presumably a good indicator of oxygen-induced lung damage.[9] GSH-P_x[10] and the HMP enzymes[11] were measured on a high-speed supernatant made from homogenised lungs.

RESULTS

The results of an experiment demonstrating the progressive increase in the activities of lung GSH-P_x, HMP and lavage protein content during oxygen

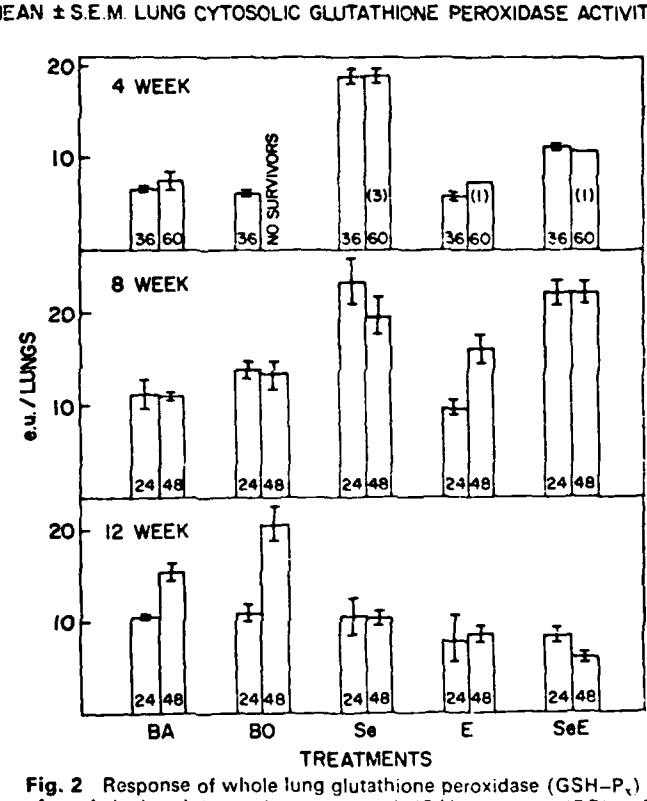


Fig. 2 Response of whole lung glutathione peroxidase (GSH-P_x) activity of rats fed a basal diet and exposed to air (BA) or oxygen (BO) or fed the basal diet plus Selenium (Se), vitamin E (E) or both (SeE) and exposed to oxygen. Numbers in the upper left hand corner of each panel indicate duration of dietary pre-treatment. *Unless otherwise indicated* in parentheses, each bar represents four observations.

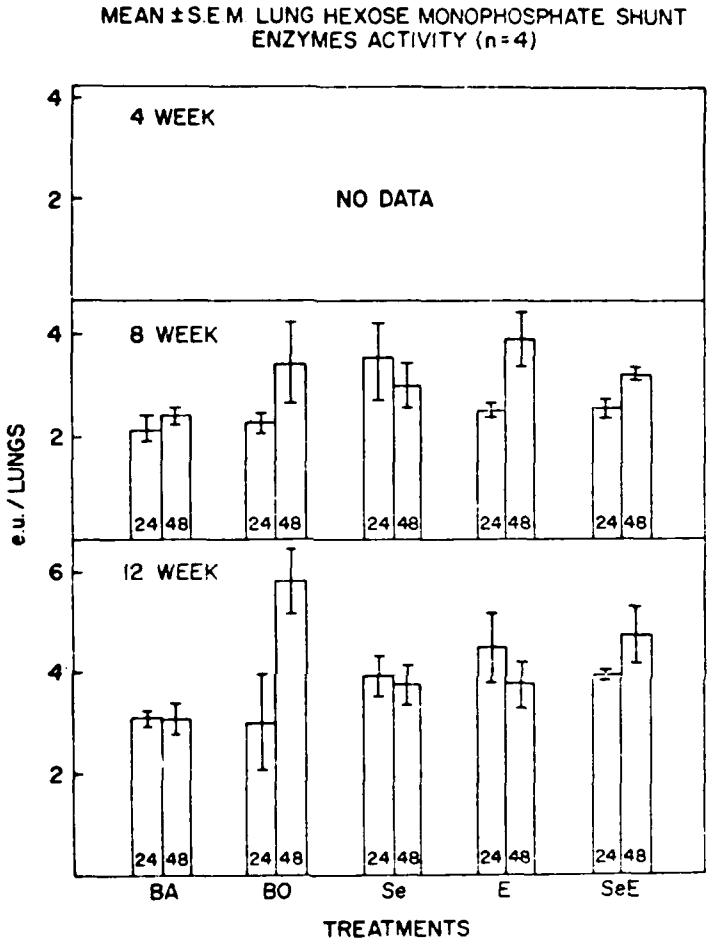


Fig. 3 Response of whole lung hexose monophosphate shunt enzymes (HMP) to oxygen exposure. See Fig. 2 caption for symbol explanation.

exposure are shown in Fig. 1. Note that GSH-P_x activity and protein content increase substantially between 24 and 48 hours, a time during which noticeable histological evidence of toxicity appears.

In diet experiments note the trend of measured parameters between 24 and 48 hours and assess the efficacy of a particular treatment based on whether or not it altered that trend. Many observed changes were statistically significant; the small number of observations precludes any definitive conclusions.

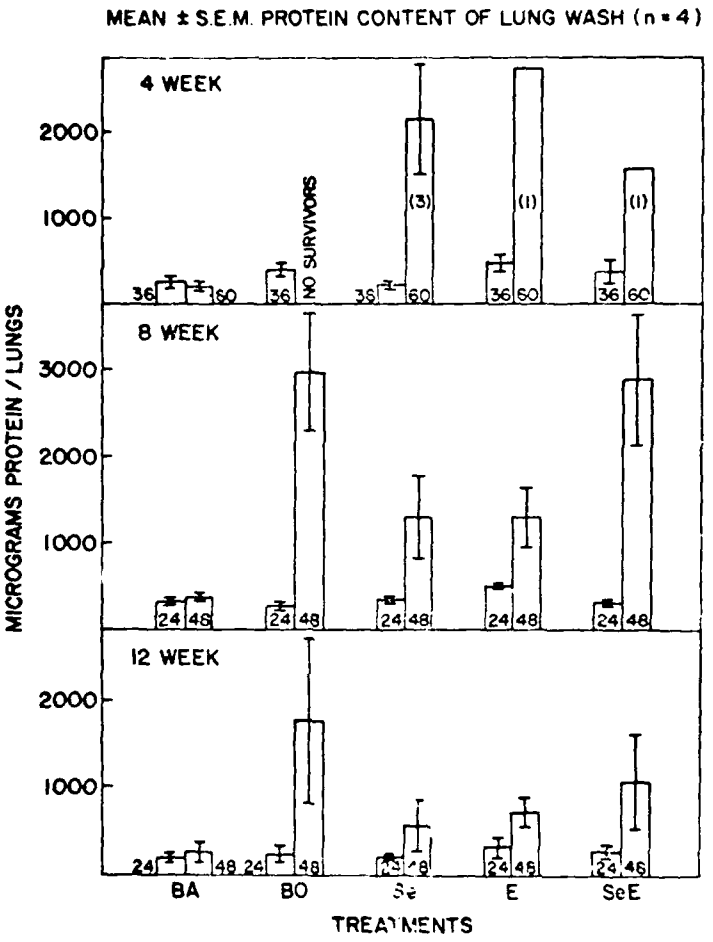


Fig. 4 Response of lung wash protein content to oxygen exposure. See Fig. 2 caption for explanation.

The results for whole lung GSH-Px are shown in Fig. 2. The high mortality after 60 hours oxygen exposure during the 4 week tests precluded parametric trend analysis. The pattern of mortality evident was consistent with trends observed during subsequent tests at 8 and 12 weeks. There were no survivors in untreated oxygen-exposed controls after 60 hours; 1 of 4 rats alive in E-fed rats with or without Se, and 3 of 4 rats alive in the Se-supplemented group. The higher

GSH-P_x levels seen in both Se-fed groups suggested induction of the enzyme by Se during the pre-exposure diet treatment period.

At 8 weeks, there was uncharacteristically no increase in enzyme activity between 24 and 48 hours in the untreated, oxygen-exposed rats, a finding contrary to those of this and other experiments. Rats fed supplemental Se again showed higher GSH-P_x activity than controls but no increase between 24 and 48 hours oxygen exposure. E-fed rats had slightly below control activity at 24 hours and a large increase in activity between 24 and 48 hours, suggesting little protection against oxygen toxicity.

After 12 weeks of feeding, all three treatment groups were protected based on no increase in enzyme activity between 24 and 48 hours when compared to untreated controls. Interestingly, the presumed Se-dependent stimulation of GSH-P_x activity in Se-supplemented rats observed after 4 and 8 weeks was absent at 12 weeks. This may be significant because it suggests that the apparent protection of Se may not have been linked to GSH-P_x activity.

The data for the HMP enzymes (Fig. 3) were consistent with those for GSH-P_x. Data for the 4 week tests were discarded for technical reasons. That for 8 and 12 weeks indicated that Se-supplemented rats were protected somewhat based on no increase in enzyme activity between 24 and 48 hours. Vitamin E supplementation apparently was not effective until 12 weeks. As with GSH-P_x, Se-fed rats tended to have higher enzyme activity at 24 hours than controls.

Figure 4 shows the data for protein content of lung lavages. At 4 weeks, those rats still alive after 60 hours oxygen exposure were clearly in worse condition than unexposed air controls. After 8 weeks, the Se- and the E-supplemented rats were somewhat protected based on the increase in alveolar protein between 24 and 48 hours; those fed both Se and E were not. After 12 weeks, all treatment groups tolerated oxygen exposure better than untreated controls.

DISCUSSION

The results suggest:

1. Dietary Se supplementation for 4 or more weeks elevates SGH-P activity of whole lung and appears to attenuate the severity of pulmonary oxygen poisoning resulting from continuous exposure at 1 ATA: the mode of action of Se is not known; besides its involvement with GSH-P_x it is also possible Se functions in electron transport.[12]
2. Dietary E supplementation offers some protection against toxicity but requires a feeding period of 12 weeks and may not be as great as that of Se. The mode of administration of E may be a factor since intra peritoneal injections exert a very dramatic protective effect.
3. There appears to be no synergistic effect between E and Se with regard to protection against toxicity although E did appear to reduce GSH-P_x activity in some cases.

The use of Se and E as prophylatic agents is experimental and requires an accurate description of dosages, duration of supplementation, and mechanism of action. However, their potential is enhanced by the fact that they are natural to mammalian systems and their biological roles are, to some degree, understood. Pursuit of this potential can lead to their use clinically and may allow safe extension of the currently accepted oxygen exposure limits.

ACKNOWLEDGMENT

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The effects of dietary supplementation with vitamin E and selenium on the response of the antioxidant enzymes during hyperoxia is described in the following paper:

Scatte, C.L. Dietary selenium and vitamin E as a possible prophylactic to pulmonary oxygen poisoning in Proc. VIth Internat. Cong. Hyperbaric Med., ed. G.E. Smith, Aberdeen University Press, 1979.

DIETARY FAT AND PULMONARY PROSTAGLANDINS

The results of an experiment in which dietary fat content was varied suggested that lipid metabolism might influence susceptibility to oxygen toxicity (Table 4).

Table 4: Mean ± SD Enzyme Activity (e.u./100 Uq Fresh Tissue) and Mortality of Rats after 72 hours Exposure to Oxygen.

Fat Content	GSH-Px	HMP	Mortality
Lab chow, 5%	.28 ± .05	.33 ± .12	1/11
Synthetic, 5%	.40 ± .10	.27 ± .03	3/10
Synthetic, 9.3%	.63 ± .12	.32 ± .04	5/11
Synthetic, 21.2%	.47 ± .13	.31 ± .04	10/11
Synthetic, 36.5%	.56 ± .13	.29 ± .01	9/10

Because dietary fat content can influence the formation of prostaglandins, substances having a range of physiological effects which might impact oxygen toxicity, we carried out a series of experiments designed to measure changes in lung prostaglandin levels during hyperoxia, the effect of dietary fat on those levels and the effect of hyperoxia on metabolism of the prostaglandins.

The following papers described those experiments:

Meydani, S.N., M.M. Mathias and C.L. Schatte. Dietary fat type and ambient oxygen tension influence pulmonary prostaglandin synthetic potential. Prostaglandins and Medicine 1:241-249, 1978.

Vader, C.R., M.M. Mathias and C.L. Schatte. Pulmonary prostaglandin metabolism during normobaric hyperoxia. Prostaglandins and Medicine, in press, 1980.

Schatte, C.L. and M.M. Mathias. Pulmonary prostaglandin metabolism during normobaric hyperoxia. in Proc. VII Symp. Underwater Physiol., ed. C.W. Shilling, in press, 1981.

Prostaglandins and Medicine 1: 241-249, 1978.

DIETARY FAT TYPE AND AMBIENT OXYGEN TENSION INFLUENCE PULMONARY PROSTAGLANDIN SYNTHETIC POTENTIAL

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ABSTRACT

Chronic hyperoxia produces pathological changes in lung which can be fatal. With an interest in delineating dietary factors which might affect the pulmonary response to hyperoxia, we fed rats a semi-synthetic diet containing polyunsaturated fatty acids (PUFA) as either 5% or 78% of the fat complement. The rats were exposed to pure oxygen at one atmosphere. Half the animals in each diet group were injected with aspirin during the hyperoxic exposure. Radioimmunoassay of lung prostaglandins (PG) $F_2\alpha$, E_2 and E_1 were performed at 0, 24, 48 and 72 hours. The major findings were: (1) Feeding the high PUFA diet elevated lung PG synthetic potential tenfold over that of low PUFA-fed animals. There was no effect of diet on mortality. (2) Hyperoxia significantly increased $F_2\alpha$-synthetic potential during the first 24 hours of hyperoxia and moderately increased the synthetic potential of E_2 and E_1. (3) Aspirin significantly depressed synthetic potential of all three PG prior to oxygen exposure but its effect was overcome during hyperoxia. Aspirin-injected rats showed 80% mortality in oxygen vs. 50% for saline controls.

We concluded that dietary PUFA and hyperoxia alter PG synthetic potential but its role in the pulmonary response to hyperoxia remains obscure.

INTRODUCTION

Chronic exposure to hyperoxia causes pulmonary edema and congestion which can be fatal (1). It has been well established that prostaglandins (PG) are intimately involved in lung function; $PGF_2\alpha$ has pulmonary vasoconstrictor activity while PGE_1 is a vasodilator in most species studied (2) including rats (3). Our interest in identifying dietary factors which might

influence the pulmonary response to hyperoxia has led us to focus on PG metabolism for the following reasons. PG synthesis is known to require oxygen and essential fatty acids. It is conceivable that a net increase in vasoconstrictor PG activity during exposure to hyperoxia might enhance the edema formation. Aspirin, a known inhibitor of PG synthesis, has been shown to exacerbate the toxic effects of hyperoxia (4). It has been demonstrated that in vitro hyperoxygenation will accelerate PG production by rat renal medulla (5) and that synthesis by at least adipose tissue and platelets can be influenced by dietary fat type (6,7). This study was conducted to investigate any changes in pulmonary PG synthetic capacity following treatment with diets containing different amounts of polyunsaturated fatty acids (PUFA) and during exposure to hyperoxia.

MATERIALS AND METHODS

One hundred-thirty-two weanling Sprague-Dawley-derived rats (Charles River, Wilmington, MA) were maintained so as to keep chronic respiratory disease at a minimum. They were fed a basal diet containing 19.0% casein, 0.4% dl-methionine, 25.0% cornstarch, 24.9% sucrose, 2.5% cellulose, 5.0% salt mix, 2.0% vitamin mix, and 21.2% fat. Half the animals were fed a diet containing primarily saturated fat (approximately 5% PUFA) as beef tallow and the other half approximately 78% PUFA as safflower oil. The diet containing beef tallow as the fat source closely approximated that consumed by the average American (8). The diets and tap water were provided ad libitum for one month, after which 18 rats from each dietary group were exposed to air and the rest were exposed to oxygen at 1 atmosphere in controlled environment chambers (9). In our model system oxygen exposure produces discernible pulmonary biochemical changes after 24 hours, incipient edema and histological symptoms at 48 hours and up to 100% mortality by 72 hours.

In order to gain insight into the possible effect of aspirin on PG synthetic capacity during hyperoxia, half of the animals in each dietary group were injected intraperitoneally with 50 mg aspirin/kg in saline at 1, 24 and 48 hours of oxygen exposure. This dose rate was determined, during a preliminary experiment, adequate to maintain a pulmonary concentration in the range of 20-100μM for 16-20 hours. That concentration range provided up to 50% inhibition of $PGF_2\alpha$, PGE_2 and PGE_1 synthesis by rat lung homogenate in vitro (unpublished results).

At 24, 48 and 60 hours, groups of animals were killed with chloroform. The left lung was homogenized in 10 volumes of 42 mM aspirin in 0.1 M potassium phosphate buffer (pH 7.4) with a teflon-pestle, glass-vessel homogenizer within 2 minutes. PG were probably synthesized during tissue manipulation so the PG content reflected the physiological potential of the tissue for PG biosynthesis. The homogenates were kept frozen for subsequent analyses for $PGF_2\alpha$, PGE_2 and PGE_1 using the radioimmunoassay described by Hwang et al (7) and McCosh, et al (10).

This method involved overnight precipitation of each prostaglandin-specific antiserum with the rabbit anti-gamma globulin (Method II). The PGE_1 antiserum had a cross-reactivity of 8±2% with PGE_2 and the PGE_2 antiserum had a cross-reactivity of 6±1% with PGE_1. The cross-reactivity with 6-keto-$PFG_1\alpha$ of the antisera for $PGF_2\alpha$, PGE_2 and PGE_1 was 2%, 0.5% and 1.0%, respectively. Parallelism was established and the recoveries of PG were shown to be 90-100%.

The results were analyzed using a 2 (oxygen response for 24 and 48 hours) x 2 (fat) x 2 (aspirin) factorial design analysis of variance. The controls and 60-hour results were analyzed by t-tests due to the unequal sample numbers. All data are presented as mean ± standard error.

RESULTS

Figures 1-3 illustrate the response of the measured PG to dietary fat treatment, aspirin injection and continuous exposure to hyperoxia. We recognize that cross-reactivity of antibodies used for radioimmunoassay precludes accurate statements of true PG concentrations; all references to PG actually indicate concentrations of $F_2\alpha$-, E_2- and E_1- like material in the assay samples. The number of observations in each group for the sample times were: 0 hours-9; 24 hours-8; 48 hours-8; 60 hours-from 1-4. The variable sample number at 60 hours of oxygen exposure resulted from an unexpectedly high mortality at that time. While dietary fat type had no effect, aspirin-injected rats experienced 80% mortality between 48 and 60 hours vs. 50% for saline-injected animals.

The most prominent effect on pulmonary PG synthetic potential was that of dietary fat type. Rats fed the high PUFA-safflower oil diet showed significantly ($p<.001$) higher levels of the three PG at all sample times than did those fed the low PUFA-beef tallow diet. The relative magnitude of the difference in saline-injected, non-oxygen-exposed controls was 8-, 13- and 11-fold for $F_2\alpha$, E_2, and E_1, respectively.

Aspirin injection significantly ($p<.001$) decreased all three PG levels in non-oxygen-exposed rats. The respective decreases for the oil-fed animals were 69% for $F_2\alpha$, 52% for E_2 and 58% for E_1, while those for tallow-fed animals were 48% for $F_2\alpha$, 25% for E_2 and 29% for E_1. These findings correspond to our observations in vitro that $F_2\alpha$ production was more sensitive to aspirin inhibition than either E_2 or E_1. Of particular interest was the fact that raising the ambient oxygen tension from the approximately 130 mmHg in air to >700 mmHg during hyperoxia attenuated the depressant effect of aspirin injection on PG synthetic potential. Hyperoxygenation appeared to be a more potent stimulator of PG synthetic potential than was aspirin a depressant.

Figure 1. Concentration of $PGF_2\alpha$-like material in rat lung homogenate.

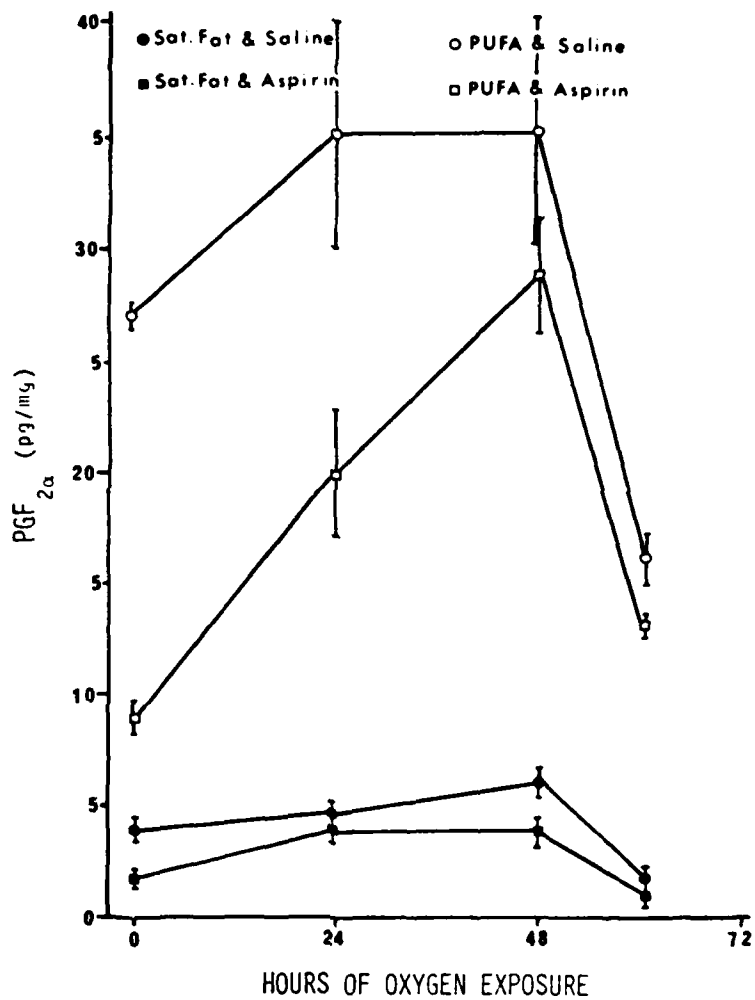


Figure 2. Concentration of PGE_2-like material in rat lung homogenate.

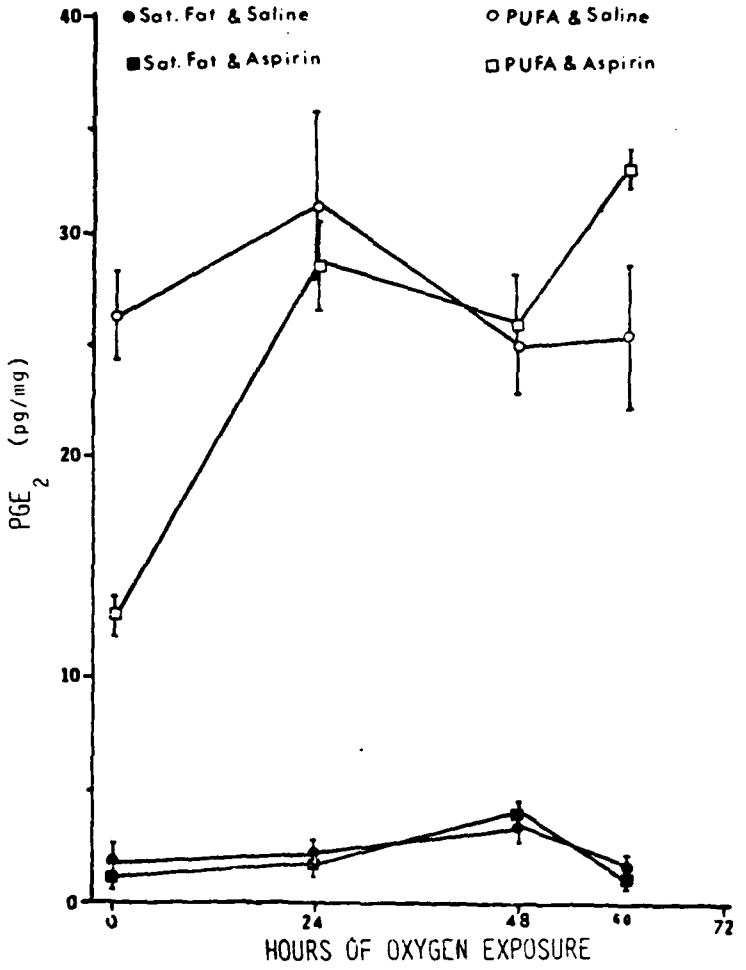
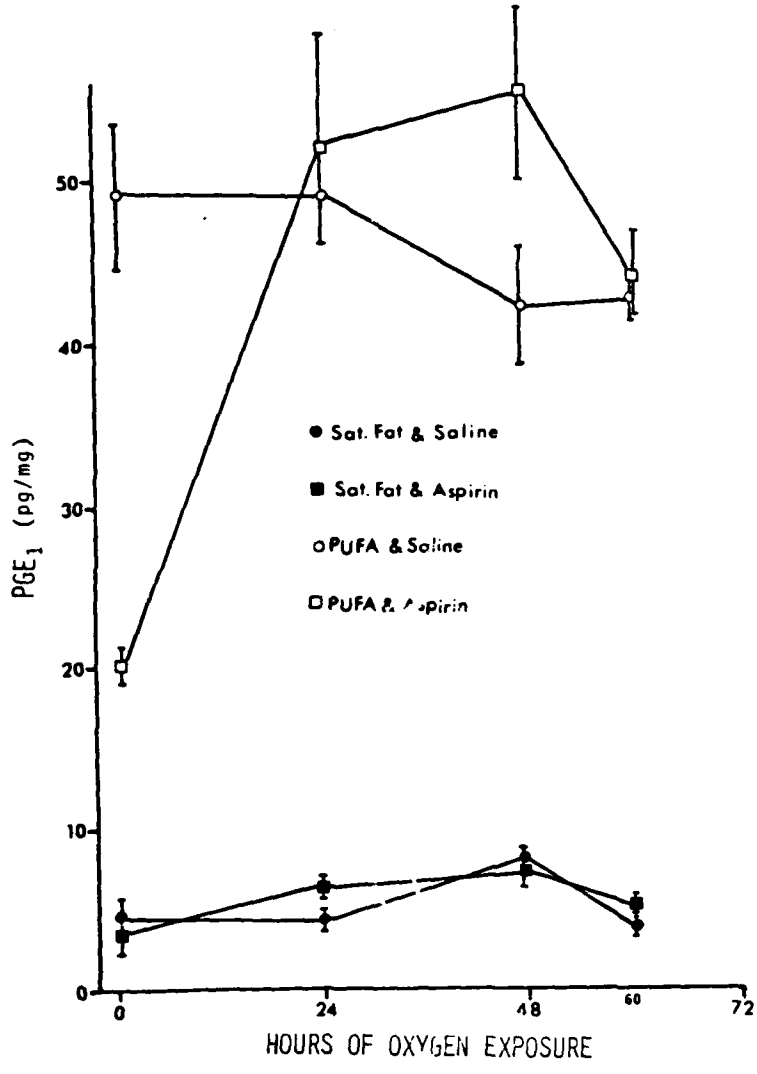


Figure 3. Concentration of PGE_1-like material in rat lung homogenate.



That hyperoxia did significantly ($p<.01$) increase $F_2\alpha$ but not E_2 or E_1 during the first 24 hours' exposure of non-aspirin-injected rats fed the safflower oil diet was a third major finding of this study. It is consonant with the report that the rate of $F_2\alpha$ synthesis by rat kidney medulla was accelerated *in vitro* when gassed with 95% oxygen (5). $PGF_2\alpha$ in the oil-fed rats significantly ($p<.05$) decreased between 48 and 60 hours, perhaps in response to the hypoxemia known to occur prior to death (11).

DISCUSSION

The results clearly demonstrate that elevated dietary PUFA and ambient oxygen tension increase the PG synthetic potential of rat whole lung. The oxygen effect was most prominent for $F_2\alpha$ and was maximal after 24 hours' exposure. If PG are involved in the pulmonary symptoms which occur during chronic hyperoxia, the changes during the first 24 hours may be critical. Few biochemical or histological changes are observed at that time (1).

Our data do not provide information concerning the *in vitro* physiological responses to the change in synthetic potential but three observations are pertinent. Administration of aspirin, an inhibitor of PG synthesis which is known to predispose patients receiving hyperoxic therapy to CNS symptoms (12), increased mortality and decreased the zero time PG synthetic potential substantially in all diet treatment groups. These two phenomena may or may not be related but the fact that aspirin inhibition of synthetic potential was overcome after 24 hours' exposure to oxygen points to the changes in PG synthetic potential during this time as both substantial and probably important.

The second point is that, despite a 10-fold increase in synthetic potential of the oil-fed rats, there was no difference in mortality between the two diet groups. While this fact seemingly suggests that PG synthetic potential is not important in the pathological response of the lung to oxygen, we believe that may not be the case. We feel rather that substantial changes in the synthetic potential of substances with powerful vasoactivity such as prostaglandins probably constitute a response of the cell to the hyperoxic stress and that the relatively crude parameter of mortality was not sufficiently sensitive to reflect more subtle differences between the two diet groups. Further experiments incorporating sensitive histological and functional parameters, particularly during the first 24 hours of hyperoxia, would provide a more definitive assessment of any effect resulting from the diet-induced changes in PG synthetic potential.

The third observation of interest is the fact that aspirin potentiation of oxygen toxicity may not be PG-related. While aspirin administration increased mortality, there was no significant difference in PG synthetic potential between the aspirin- and saline-injected animals after 24 hours of oxygen exposure.

Either the aspirin effect does not involve PG or the lower PG synthetic potential of aspirin-injected rats prior to and during the first 24 hours of hyperoxia was critical.

It is possible that PG-related factors other than synthetic potential might explain the results. Recently-discovered intermediates and other products such as thromboxane A_2 or PGI_2 are physiologically more potent than the PG we measured (12) and may have been responsible for our results. The rate of PG catabolism may have been altered since PG dehydrogenase has been reported to be inhibited by hyperoxia (14). Perhaps the rate of PG conversion to less potent substances is more crucial than synthetic potential or endogenous levels at any given time. The lungs' sensitivity to hyperoxia may well be related to any impairment of its central role in PG catabolism.

Whatever the mechanisms involved, this study suggests that both dietary fat type and ambient oxygen tension are factors pertinent to pulmonary prostaglandin synthesis. To the clinician utilizing dietary fat alterations or oxygen therapy as treatment, the potential consequences of increased prostaglandin synthesis must be considered. To the basic scientist working with prostaglandin synthesis, our results indicate that endogenous substances (PUFA and oxygen) may be controlling factors of the lung's PG synthetic potential. We are further investigating these possibilities.

ACKNOWLEDGMENT

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PULMONARY PROSTAGLANDIN METABOLISM DURING NORMOBARIC HYPEROXIA

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ABSTRACT

Prostaglandin metabolism by rat lung tissue was measured following exposures of 6, 24 and 48 hours to either pure oxygen or air at one atmosphere. Tissue concentrations of PGE_1, PGE_2 and $PGF_{2\alpha}$ were not altered by oxygen exposure. Prostaglandin synthetase activity decreased between 24 and 48 hours but was not significantly different from control at 48 hours. Combined prostaglandin dehydrogenase/reductase activity decreased between 24 and 48 hours to 13% of control values and was significantly lower than in air at 48 hours. The plasma concentration of 13,14 dihydro-15-keto $PGF_{2\alpha}$, a catabolite of $PGF_{2\alpha}$, was significantly lower in oxygen-exposed rats at 24 and 48 hours. We conclude that endogenous pulmonary prostaglandin concentrations are maintained during hyperoxia but that catabolism of prostaglandins by the lungs may be impaired.

INTRODUCTION

Exposure to oxygen at an ambient pressure of one atmosphere produces pulmonary pathology characterized by congestion, edema, capillary endothelial cell necrosis and consolidation (1). While no relationship between prostaglandins (PG) and pulmonary oxygen toxicity has been demonstrated, several observations suggest such a possibility.

First, molecular oxygen is a requirement for PG synthesis. If it is a rate-limiting reactant, increasing available oxygen might increase PG production. Consistant with this possibility, production of PGE and PGF series was enhanced by hyperoxia in rat renal medullary slices (2) and lung tissue homogenates (3).

Second, one of the mechanisms by which hyperoxia is thought to exert deleterious effects on cells is through accelerated production of peroxides (4). Increased peroxide tone has been reported to enhance the activity of PG synthetase (5).

Third, aspirin, an inhibitor of PG synthetase, exacerbated convulsions induced by oxygen at high pressure (6) and increased mortality during oxygen exposure at one atmosphere (3). Indomethacin, another inhibitor of PG synthesis, partially attenuated the decrease in pulmonary vascular resistance observed during hyperoxia (7) but did not affect the mortality of rats exposed to normobaric hyperoxia (8). The vasoactivity of selected PG has been shown to vary as a function of oxygen tension (9).

Finally, there is evidence that the endothelial cells of the pulmonary vasculature are the first to show signs of necrosis resulting from oxygen toxicity (10). Vascular endothelial cells have also been identified as a primary site of synthesis of prostacyclin, or PGI_2, one of the most potent vasodilators and anti-aggregating agents in the circulation (11).

These observations present the possibility that aberrations in PG metabolism during oxygen exposure might be associated, causally or otherwise, with the vascular pathology which produces the known symptoms of oxygen poisoning.

This study was undertaken to profile plasma and lung tissue concentrations of selected PG and the activities of key enzymes during exposure to normobaric hyperoxia.

METHODS

We performed two similar experiments which differed primarily in duration of exposure to hyperoxia. In both, chronic disease-free male Sprague-Dawley rats (Charles River Co.) weighing approximately 180g were individually caged in a room maintained at 22°C and 40% relative humidity. Water and a semi-synthetic diet (3) were available ad libitum. The diet composition was similar to that consumed by the average American, containing 40% of calories as fat with a polyunsaturated: saturated fatty acid ratio of 0.7.

After 10 days of diet feeding, the animals were randomly divided into groups for exposure either to air or pure oxygen at one atmosphere. Exposures were carried out in converted autoclaves with a volume of 300 liters. Ambient temperature was maintained at 25-27°C by means of a water jacket. Carbon dioxide concentration was held at <0.5% by adjusting gas flow through the chamber. Animal waste dropped through the floor grid onto absorbent mixed with boric acid to reduce the buildup ammonia. Diet and water were available ad libitum throughout the exposure.

Animals were sacrificed by decapitation after 0, 6 or 24 hours of exposure in the first experiment and after 6, 24 or 48 hours exposure in the second. About 10ml of blood was collected in polypropylene cups containing final concentrations of 1 μM indomethacin and 10 mg EDTA to stop PG synthesis. Blood was mixed during collection to prevent clotting and then kept on ice.

Lungs were excised with a minimum of mechanical stress and processed for determination of endogenous PG concentrations or the activities of PG synthetase and combined PG dehydrogenase/reductase as follows.

Plasma and Tissue Prostaglandins

Blood was spun at 40,000g for 20 minutes at 4°C to obtain platelet-poor plasma, which was then adjusted to pH 3.8 ± 0.2 with 0.1M citrate. Tissues which had been quick-frozen in liquid nitrogen at sacrifice were homogenized 1:10 w/v in 0.1M citrate-phosphate buffer, pH 3.8, containing 1 mM indomethacin.

All samples were extracted twice with 5 volumes ethyl acetate for 45 minutes on a horizontal shaker. Recoveries ranged from 87-95%. The extracts were combined and dried under nitrogen, reconstituted to 2.5ml with buffer and radioimmunoassayed for PGE_1, PGE_2, $PGF_{2\alpha}$ and 13,14 dihydro-15-keto $PGF_{2\alpha}$ ($mPGF_{2\alpha}$), the major catabolite of $PGF_{2\alpha}$.

Prostaglandin Synthetase

Freshly-excised lung tissue was homogenized on ice 1:5 w/v in 0.25M sucrose containing 1mM EDTA and 0.1M Tris buffer, pH 7.4. Homogenates were centrifuged at 9000g for 20 minutes at 4°C, the pellet removed, then recentrifuged at 100,000g for 60 minutes at 4°C. The microsomal pellet was removed, the supernate boiled for 5 minutes and the denatured protein removed by centrifuging at 7000g for 15 minutes. Protein concentration was determined by the microbiuret method (12) and the microsomal suspension adjusted to a protein concentration of 10mg/ml.

Enzymatic activity was measured using a modification of the method of Parkes and Eling (13). The following mixture was incubated at 37°C for two minutes: 1.0ml 0.1M Tris buffer, pH 9.0, 100 μl 1.0mM $CuSO_4$, 100 μl 20mM dithiothreitol, 500 μl boiled supernate, 100 μl of 0.643 M arachidonic acid and 100 μl of the microsomal suspension. The reaction was stopped by

adding 50 µl 1N HCl. Excess arachidonate was immediately removed by extraction with 10ml hexane. Sample pH was adjusted to 7.0 with 50 µl 1N NaOH. The amount of product generated during the incubation was determined by radioimmunoassay for $PGF_{2\alpha}$. Blanks contained 100 µl buffer instead of the microsomal preparation.

Combined Prostaglandin Dehydrogenase/Reductase (PGDH/R)

Freshly-excised lung tissue was homogenized on ice 1:10 w/v in 0.092M potassium phosphate buffer, pH 7.3, containing 4mM $MgCl_2$ and 0.1mM dithiothreitol. The homogenates were centrifuged at 10,000g for 20 minutes, the pellet removed, and recentrifuged at 78,000g for 60 minutes at 4°C. Protein concentration in the supernatant was determined by microbiuret and adjusted to 10mg/ml.

Enzyme activity was determined using modifications of the methods of Parkes and Eling (14) and Lee and Levine (15). The following mixture was incubated at 37°C for 40 minutes: 400 µl 0.25M potassium phosphate buffer, pH 8.0, 100 µl 8mM NAD, 100 µl 20 µg/ml $PGF_{2\alpha}$ and 100 µl supernatant. Following the incubation, 100 µl 32mM NADH was added to inhibit PGDH and serve as a cofactor for the reductase. The incubation was continued for 20 minutes, then stopped by adjusting the pH to 3.0 with 100 µl 1.5N HCl and placing the reaction tubes on ice. Prior to radioimmunoassay for 13,14 dihydro-15-keto $PGF_{2\alpha}$, sample pH was adjusted to 7.0 with 100 µl 1N NaOH. Blanks contained 100 µl buffer in lieu of the enzyme preparation.

Radioimmunoassay

Double antibody sequential precipitation radioimmunoassays (Method I) were done under equilibrium conditions at 4°C (16). Antisera for PGE and $PGF_{2\alpha}$ was obtained from Research Products International, Elks Grove Village, IL; for PGE_2, from Sioma Chemical Co., St. Louis, MO; and for 13,14-dihydro-15-keto $PGF_{2\alpha}$ from J. D. Dupont and M. M. Mathias, Department of Food Science and Nutrition, Colorado State University. Cross reactivities are shown in Table 1. Recoveries of added PG ranged from 90-104% and parallelism was established for all assays. Interassay precision expressed as th coefficient of variation (n=6) was 6.6% for PGE_1, 3.0% for PGE_2, 7.8% for $PGF_{2\alpha}$ and 8.8% for $mPGF_{2\alpha}$.

Table 1. Specificity of the prostaglandin antisera used in the radioimmunoassays.

Prostaglandin	Antisera			
	PGE_1[a]	PGE_2[a]	$PGF_{2\alpha}$[a]	$mPGF_{2\alpha}$[b]
PGE_1	100.0	1.8	< 4.0	1.3
PGE_2	4.3	100.0	< 3.0	0.2
$PGF_{1\alpha}$	< 0.1	< 0.1	< 3.0	< 0.1
$PGF_{2\alpha}$	< 0.1	< 0.1	100.0	0.1
$PGF_{2\alpha}$[b]	NA[c]	NA	< 3.3	NA
$mPGF_{2\alpha}$[b]	< 0.1	NA	2.5	100.0
$mPGE_2$[b]	< 0.1	0.1	NA	2.3
$mPGE_1$[b]	0.1	< 0.1	NA	1.1
Thromboxane B_2	NA	NA	NA	< 0.1
Arachidonate	< 0.01	NA	< 0.01	< 0.01

[a] Data obtained from supplier.
[b] 13,14-dihydro-15-keto PG; data from M. M. Mathias.
[c] Not available.

RESULTS

In the first experiment, there were no significant changes in lung tissue PG concentrations or the circulating metabolite of $PGF_{2\alpha}$ (Table 2). Lung tissue $mPGF_{2\alpha}$ levels tended to increase with oxygen exposure while $PGF_{2\alpha}$ decreased but it is unclear whether or not these were related.

Table 2. Effect of air or oxygen on lung and plasma prostaglandin concentrations (Experiment I).

Hours of Exposure	Lung				Plasma
	PGF_2	PGE_1	PGE_2	$mPGF_{2\alpha}$	$mPGF_{2\alpha}$
	(ng/100 mg wet wt.)				(ng/ml)
0					
air	6.5 ± 5.8[a]	4.4 ± 2.5	2.4 ± 1.5	2.2 ± 0.8	3.9 ± 0.8
6					
air	3.1 ± 2.2	5.2 ± 1.8	2.3 ± 1.4	3.5 ± 0.5	12.8 ± 4.1
oxygen	3.8 ± 3.4	3.6 ± 1.8	1.1 ± 0.3	3.4 ± 2.6	9.5 ± 0.8
24					
air	3.6 ± 1.9	6.7 ± 4.8	1.6 ± 0.9	3.4 ± 0.6	4.7 ± 1.6
oxygen	1.4 ± 0.7	2.5 ± 0.8	1.0 ± 0.6	2.8 ± 0.6	6.2 ± 1.0

[a] Mean ± S.E.M., n = 6.

In the second experiment (Table 3), tissue PG concentrations were again unaffected by oxygen but plasma $mPGF_{2\alpha}$ was significantly reduced in oxygen-exposed rats after 24 and 48 hours. This presented the possibility that inactivation of $PGF_{2\alpha}$ might have become impaired. Consistent with this result, Crutchley et al. (17) recently reported that $mPGF_{2\alpha}$ release from isolated-perfused guinea pig lungs was depressed following exposure to hyperoxia.

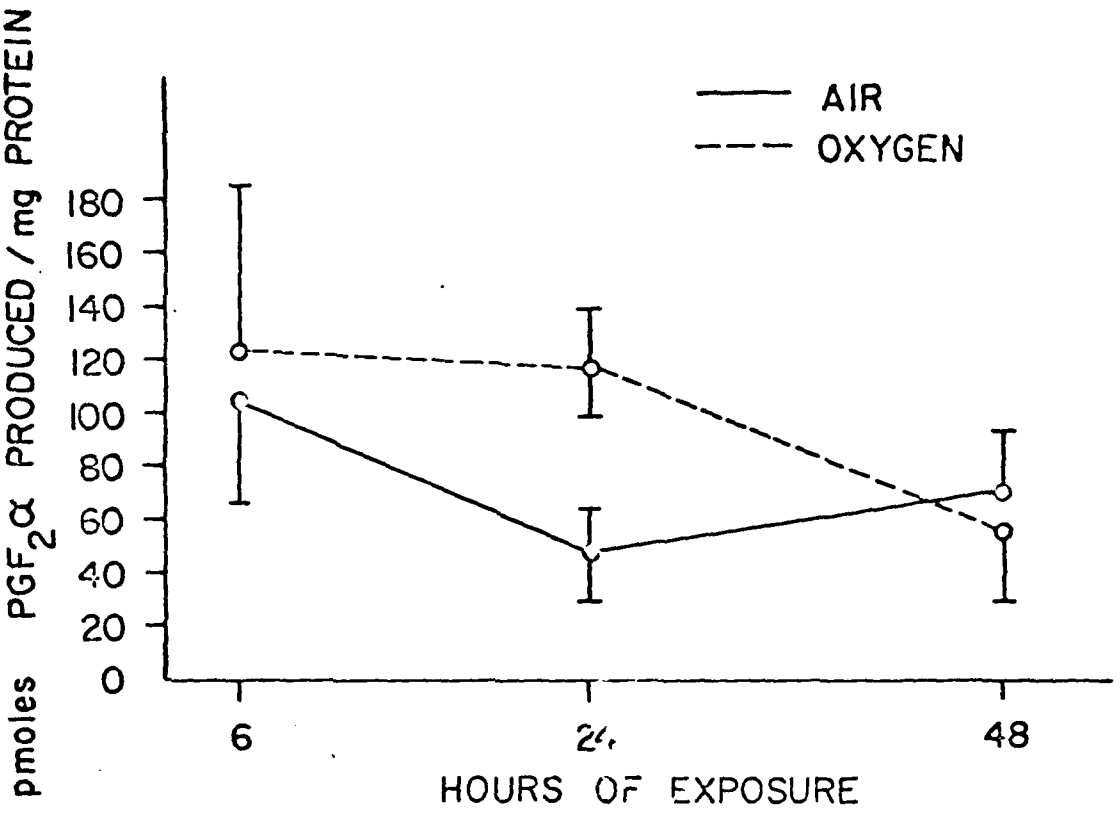
Table 3. Effect of air or oxygen on lung and plasma prostaglandin concentrations (Experiment II).

Hours of Exposure	Lung			Plasma
	PGE_1	PGE_2	$PGF_{2\alpha}$	$mPGF_{2\alpha}$
	(ng/100 mg wet wt.)			(ng/ml)
6				
air	1.7 ± 0.7[a]	0.55 ± 0.20	7.2 ± 2.6	NA[b]
oxygen	1.8 ± 0.9	0.68 ± 0.20	5.8 ± 2.3	NA
24				
air	1.2 ± 0.3	0.38 ± 0.20	4.7 ± 0.7	4.0 ± 0.3
oxygen	1.7 ± 0.6	0.61 ± 0.20	5.4 ± 1.9	2.2 ± 0.6[c]
48				
air	1.3 ± 1.5	0.51 ± 0.30	4.3 ± 3.5	4.1 ± 0.8
oxygen	0.9 ± 0.3	0.52 ± 0.20	4.1 ± 0.9	2.6 ± 0.5[d]

[a] Mean ± S.E.M., n = 6.
[b] Not available.
[c] $p<.001$.
[d] $p<.03$.

The activity of PG synthetase declined significantly ($p<.001$) between 24 and 48 hours (Figure 1) of oxygen exposure, but was not significantly different from air control values at that time. This is consistent with the findings of Parkes and Eling (14) who observed no difference in synthetase activity in guinea pig lung after a similar exposure. PGDH/R activity also decreased significantly at 48 hours ($p<.001$) to 13% of the activity in air controls. Our results are quantitatively similar to the 70% decline in activity of PGDH observed in guinea pig lungs after 48 hours of hyperoxia (14) and an 85% decrease seen in the same species after 72 hours (17). Thus, the potential for normal rates of synthesis but lesser rates of degradation existed in oxygen-exposed animals although we cannot say that the actual rates of synthesis or catabolism were altered *in vivo*.

Figure 1. Mean ± S.E.M. Lung Prostaglandin Synthetase Activity (n = 4-8).

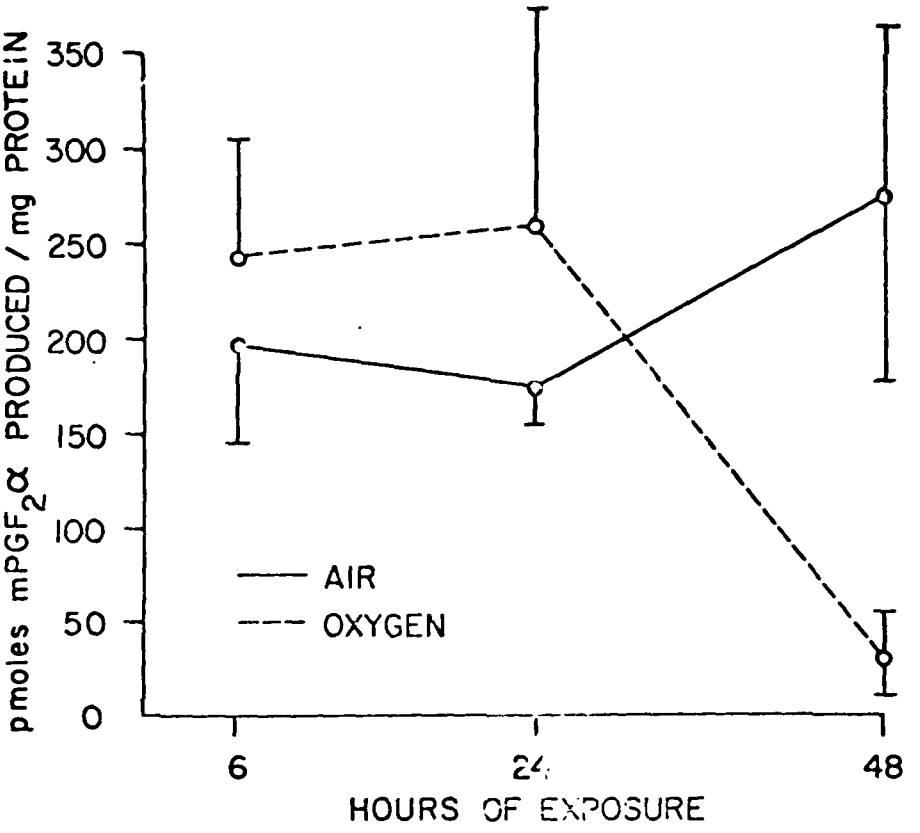


DISCUSSION

Our results indicate that lung tissue levels of PGE and PGF are maintained during hyperoxia, but that the *in vivo* rate of catabolism could decline between 24 and 48 hours of exposure. The fact that PGDH/R activity is reduced by oxygen is consistent with the established premise that enzymes containing sulfhydryl groups are susceptible to oxidant damage (4). The dehydrogenase, but not the reductase, has sulfhydryl groups (18).

If depressed PGDH/R activity actually reduced degradation of PG, reduced circulating levels of PG metabolites might logically result. We chose to measure the metabolite of $PGF_{2\alpha}$ on the assumption that its production by PGDH/R would be representative of the relative production of other PG metabolites converted by the enzyme complex. $mPGF_{2\alpha}$ levels in the plasma were significantly lower at 24 and 48 hours, although it is not certain that this resulted from any change in the activity of PGDH/R. In only the second experiment was circulating metabolite concentration significantly lower after 24 hours of hyperoxia and at a time when activity of the enzyme complex was not depressed.

Figure 2. Mean + S.E.M. Lung Combined Prostaglandin Dehydrogenase/Reductase Activity (n = 4-8).



The timing of these changes in PG metabolism are intriguing in light of the chronology of known pathological symptoms associated with pulmonary toxicity. Our rat model tolerates normobaric hyperoxia well for 24 hours. Up until that time, no gross symptoms or histological abnormalities are observed. The activities of key antioxidant enzymes, such as glutathione peroxidase, glucose-6-phosphate dehydrogenase and superoxide dismutase, do not change appreciably (19) suggesting that the cells are not yet responding to oxidant stress. By 48 hours, histological evidence of edema is apparent. Thereafter, the animals' condition deteriorates rapidly. Respiratory distress, anorexia, weight loss, intrapleural edema and gross histological pathology occurs. Pulmonary antioxidant enzyme activity decreases (18). Mortality ranges from 50-100% by 72 hours.

It therefore seems likely that one or more biochemical events occurring between 24 and 48 hours precedes, and perhaps triggers, the onset of overt toxicity. We believe that an alteration in the metabolism of PG, particularly a decrease in their catabolism, may be one of those changes.

Our data do not address the question of mechanism. But one possibility is that an imbalance of vasoconstrictor and vasodilator substances might alter pulmonary vascular pressure and perhaps capillary integrity. Yam and Roberts (7) found that hyperoxia caused a decreased pulmonary vascular resistance in neonatal swine. The effect was attenuated by administration of the PG synthetase inhibitor, indomethacin. If, for instance, catabolism of dilator PG were selectively impaired more than that of constrictor PG, the resulting vasodilatation might produce or exacerbate the congestion and vascular stasis seen in the early stages of pulmonary toxicity. Prostaglandins are also involved in the integrity of cells through mediation of ionic transport and calcium concentrations (20). If an alteration of PG metabolism compromised the integrity of pulmonary capillary cells, edema formation might be triggered or enhanced.

The field of prostaglandin pharmacology is currently ripe with new discoveries of more prostaglandin and thromboxane substances, many of which may be more important than those measured in this study. For example, hyperoxia was recently reported to enhance the release of TxB_2 in sensitized isolated-perfused guinea pig lungs (17); we have preliminary evidence that synthesis of this potent vasoconstricter and platelet-aggregating agent is similarly increased by hyperoxia *in vivo*. We are pursuing the question of the relationship between the decrease in PGDH/R activity and the onset of pulmonary symptoms as well as any possible role newly-discovered prostaglandins and thromboxanes may have in pulmonary oxygen toxicity.

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PULMONARY PROSTAGLANDIN METABOLISM DURING NORMOBARIC HYPEROXIA

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INTRODUCTION

We have evaluated the effect of several dietary constituents on the incidence and time course of pulmonary oxygen toxicity. The most striking alteration of relative susceptibility to pulmonary toxicity was produced by varying dietary fat content (Table I). Since two antioxidant enzymes known to increase their activity as part of the cellular response to oxidant gases (1,2) were unaltered by the diets, we looked for some other factor which could have caused the differential response to hyperoxia. The ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids, P/S ratio, was held constant as fat content was increased in this experiment. We therefore wondered if the differential content of the PUFA, linoleic acid, a precursor of prostaglandins (PG) and thromboxanes (Tx), might account for the differences in mortality.

Table I. Mean ± SD pulmonary activities (*e.u./100 mg fresh tissue*) of pulmonary antioxidant enzymes in rats fed diets containing various amounts of fat calories and mortality after 72 hours exposure to normobaric hyperoxia.

Fat Content[a]	GSH-Px[b]	G6PDH[c]	Mortality
Lab chow, 5%	.28	.33	1/11
Semi-synthetic, 5%	.40	.27	3/10
Semi-synthetic, 9%	.63	.32	5/11
Semi-synthetic, 21%	.47	.31	10/11
Semi-synthetic, 36%	.56	.29	9/10

[a] Percent by weight. P/S ratio was 0.7 for all diets.

[b] Glutathione peroxidase.

[c] Glucose-6-phosphate dehydrogenase.

We have subsequently performed experiments to investigate the effects of dietary fat content on pulmonary PG synthesis, and any changes in PG metabolism which might occur during hyperoxic exposure. Our intention is to develop a dietary regimen optimum for tolerating a potentially toxic oxygen exposure.

METHODS AND RESULTS

Effect of PUFA on Lung PG Profiles:

We demonstrated that endogenous levels of PGE_1, PGE_2, PGF_2 and PGI_2 can be altered in rat lung by varying P/S ratio of the dietary fat. Table II shows an upward trend of PG concentrations as P/S ratio rises. However, marked differences occurred only with the diet containing the highest PUFA level. All experiments reported in this paper utilized a semi-synthetic diet which approximates that of the average American male. It contained, by weight, 21% fat, 19% balanced protein, 50% carbohydrate, 2.5% fiber and nutritionally adequate amounts of vitamins and minerals. In all experiments, weanling male Sprague-Dawley rats (CDF-1 strain, Charles River Co.) were fed the diets for 2-4 weeks prior to the experimental protocol. Prostaglandin analyses were done by radioimmunoassay as previously described (3).

Four observations are pertinent. First, the increase in PG concentrations with increasing P/S ratio was probably due to increasing amounts of linoleic acid. By weight, beef tallow contains about 2.5%, soybean oil about 50% and safflower oil about 75% linoleate. Second, the relative magnitude of the concentrations of PG may have biological significance. PGF_2 , a pulmonary

Table II. Endogenous prostaglandin concentrations (ng/g fresh tissue) in rat lung as a function of P/S ratio.

Fat type[a]	P/S ratio	PGF_2	PGE_2	PGE_1	TxB_2[b]	6-keto-PGF_1 [b]
Fat-free	0.00	21.3	3.4	22.7	9.2	215
Beef tallow	0.05	26.9	6.8	28.3	9.3	253
Beef tallow/ soybean oil	0.55	26.3	4.8	29.6	11.9	359
Soybean oil	5.50	40.9	5.0	39.5	18.8	332
Safflower oil	10.32	91.1[d]	13.6[d]	69.8[d]	26.2[d]	798[d]

[a] 21% of diet, by weight.

[b] spontaneous degradation product of TxA_2.

[c] spontaneous degradation product of PGI_2.

[d] significant ($P<0.05$ or better) effect of dietary fat.

vasconstrictor (4), and PGE_1, a vasodilator (4), are in about equal concentration. It is interesting to speculate whether or not vascular resistance might be influenced by a specific balance between the two. If so, an imbalance in the concentrations or activities of the two substances could play a role in the vascular stasis and congestion which characterizes the toxic stage of hyperoxia. Third, TxA_2 is a potent vasoconstrictor and platelet aggregator which has been implicated in pulmonary hypertension (5). While its concentration is relatively low, it increased with increasing dietary fat content. It is possible that the correlation between fat content and mortality may have been associated with pulmonary hypertension. Fourth, PGI_2 is one of the most

potent vasodilators and inhibitors of platelet aggregation yet discovered (6). Its concentration in the lung was ten times that of the other PG. The implications for oxygen poisoning of this high concentration are uncertain but bear further scrutiny.

Effect of Hyperoxia and Aspirin on Lung PG Profiles

PG concentrations might reasonably be expected to be altered by hyperoxia, and possibly play a role in the pulmonary response to it, because oxygen is required for PG synthesis. Consistent with this, PG synthesis *in vitro* by kidney slices was significantly enhanced by hyperoxygenating the medium (7).

If PG profiles do change during hyperoxia, it might be possible to alter the lungs' response to hyperoxia by inhibiting their synthesis. Aspirin, which inhibits the rate-controlling synthetase enzyme, has been reported to exacerbate the toxic central nervous system effects attendant upon hyperbaric oxygen exposure (8). But indomethacin, another synthetase inhibitor, did not alter mortality rates of rats during normobaric hyperoxia (9).

We fed rats the semi-synthetic diet containing either 21% beef tallow or safflower oil for three weeks and then exposed them to normobaric hyperoxia for up to 60 hours (3). Half the animals in each diet group were injected intraperitoneally with aspirin during each day of the oxygen exposure, at a dose determined during preliminary experiments to provide 50% inhibition of PG synthesis. Animals were sacrificed at 0, 24, 48 and 60 hours and the lungs analyzed for PGE_1, PGE_2 and PGF_2 .

Figure 1 shows the results for PGF_2 which are representative of the pattern seen for the other PG. The safflower oil diet stimulated PG synthesis to levels about ten times those in the tallow-fed rats. Aspirin depressed synthesis of all the PG prior to oxygen exposure in both diet groups. But hyperoxia powerfully stimulated synthesis even in the presence of aspirin. While diet had no effect on mortality at 60 hours, aspirin-injected rats had an 80% death rate versus 50% for saline controls at that time.

The fact that the safflower oil diet markedly altered PG synthesis but did not alter mortality suggests that concentration changes of at least the PG measured here do not appear to play a role in the toxic response. We now believe that TxA_2 and PGI_2, for which we did not have assays at the time of this experiment, may have been responsible for the effects observed rather than the PGE and PGF series measured. Aspirin clearly can enhance the toxicity although it is not certain that its effect on PG synthesis is the mechanism.

Effect of Oxygen on PG Synthesis and Degradation

We next wished to ascertain whether or not hyperoxia altered the activity of PG synthetic or degradative enzymes in rats. We had assumed that hyperoxia increased PG concentrations by providing substrate, O_2. However, Parkes and Eling (10) reported that oxygen exposure did not alter the activity of PG synthetase but did decrease that of PG dehydrogenase in guinea pigs. They suggested that degradation of circulating PG by the lungs might be impaired during prolonged oxygen exposure. Studies using isolated-perfused lung preparations have shown that clearance of infused PG is indeed reduced in lungs

exposed to hyperoxia (11,12). If degradation were impaired, PG levels might increase without a change in rate of synthesis.

Rats were fed the semi-synthetic diet with a P/S ratio of 0.7, then exposed to air or oxygen at atmospheric pressure for 0, 6, 24 or 48 hours. Lung concentrations of PGE_2, PGF_2, TxB_2, 6-keto-PGF_1 and 13,14-dihydro-15-keto PGF_2 ($mPGF_2$) the metabolite of PGF_2 were assayed. Plasma levels of $mPGF_2$ were also measured as an indicator of clearance of PGF_2 from the blood by the lungs. This metabolite assay was the only one available at the time of the experiment. It was assumed that it would be representative of the clearance of other PG from the blood. In addition, the activities of PG synthetase and the PG dehydrogenase/ reductase (PGDH/R) complex were measured in pulmonary tissue as previously described (13).

Figure 2 shows that synthetase activity was not altered by oxygen exposure (13), suggesting that synthetic capacity was not altered. PGDH/R activity decreased substantially between 24 and 48 hours of oxygen exposure (Figure 3). In Table III, it may be seen that circulating levels of $mPGF_2$ declined progressively during hyperoxia and were significantly different from both zero time and air-exposed controls at 48 hours. Thus, our <u>in vivo</u> results are supportive of the previous findings in isolated lungs with regard to PG clearance during hyperoxia.

Concentrations of PGE_2 and TxA_2 increased with oxygen exposure but were not significantly different from air-exposed controls. The other measured PG did not change during the exposures. The absence of systematic changes in endogenous PG concentration and the decline in circulating metabolite during

hyperoxia confirm our previous results (13). These results suggest that pulmonary PG synthesis during oxygen exposure is not markedly enhanced when the rats are fed a mixed fat diet. They also suggest that an apparent change in PG degradation does not significantly change endogenous pulmonary PG concentrations.

Table III. Plasma $mPGF_2$ (ng/ml) and pulmonary prostaglandin (ng/g fresh tissue) concentrations of rats exposed to normobaric hyperoxia.

	Plasma			Lung		
Hyperoxia (hours)	$mPGF_2$	PGE_2	PGF_2	$mPGF_2$	TxB_2[a]	6-keto-PGF_1[b]
0	14.5	5.4	40.6	14.5	6.7	74.8
6	9.8	7.5	29.7	17.6	9.1	113.6
24	10.1	11.7	48.1	30.7	10.1	133.0
48	6.8[c]	12.3[c]	42.9	27.9	12.5[c]	110.0

[a] Spontaneous degradation product of TxA_2.

[b] Spontaneous degradation product of PGI_2.

[c] Significant ($P \leq 0.05$ or better) effect of time.

DISCUSSION

PG Degradation and Oxygen Toxicity

We cannot yet say whether or not the decline in activity of PGDH/R and levels of circulating $mPGF_2$ are causally-related to the development of pulmonary toxicity. But they coincide chronologically with the appearance of visible symptoms of that toxicity. Our rat model tolerates hyperoxia well for 24 hours; no change in the activity of antioxidant enzymes (14) or histopathology is evident at that time. Between 24 and 48 hours, one or more biochemical

events occur to produce visible symptoms of dyspnea, pulmonary hemorrhage and intrapleural edema at 48 hours. Mortality is close to 100% by 72 hours. The declining activity of PGDH/R and an impaired ability to remove circulating PG could be one of the critical biochemical events.

Possible Mechanisms of PG

Although PG involvement in pulmonary oxygen poisoning is not yet proved, there are at least two ways in which they might play a role. All of the PG are vasoactive and at least PGI_2 and TxA_2 influence platelet aggregatibility (15). It is possible that alterations in the vasoactivity of one or more PG during hyperoxia contribute to the vascular congestion and edema which characterize toxicity.

A second possibility hinges on the fact that the integrity of the pulmonary capillary endothelium is compromised. The vasodilator PGI_2 is probably synthesized by these cells (16). And it is endothelial cells which seem most susceptible to hyperoxia, undergoing death and necrosis prior to the onset of edema (17). If PGI_2 synthesis were impaired as a result, changes in pulmonary vascular pressure might contribute to the edema. Alternatively, it may be an aberration of PG production which initiates endothelial cell necrosis since PG are involved in membrane transport and intracellular functions (18).

Clinical Implications of PG in Pulmonary Oxygen Poisoning

If PG prove to play a part in the etiology of oxygen toxicity, manipulation of their concentrations and activity by the use of diet and drugs might allow reduction of the occurrence and severity of symptoms. Several drugs with

demonstrated efficacy in altering PG synthesis are already used clinically to treat platelet hyper-aggregability (19). Our results thus far suggest that PG synthesis might also be altered by manipulation of dietary fat content. Further definition of any role these important and ubiquitous substances might have in preliminary toxicity includes the promise of useable clinical treatments to reduce the detrimental effects of normobaric hyperoxia.

ACKNOWLEDGMENT

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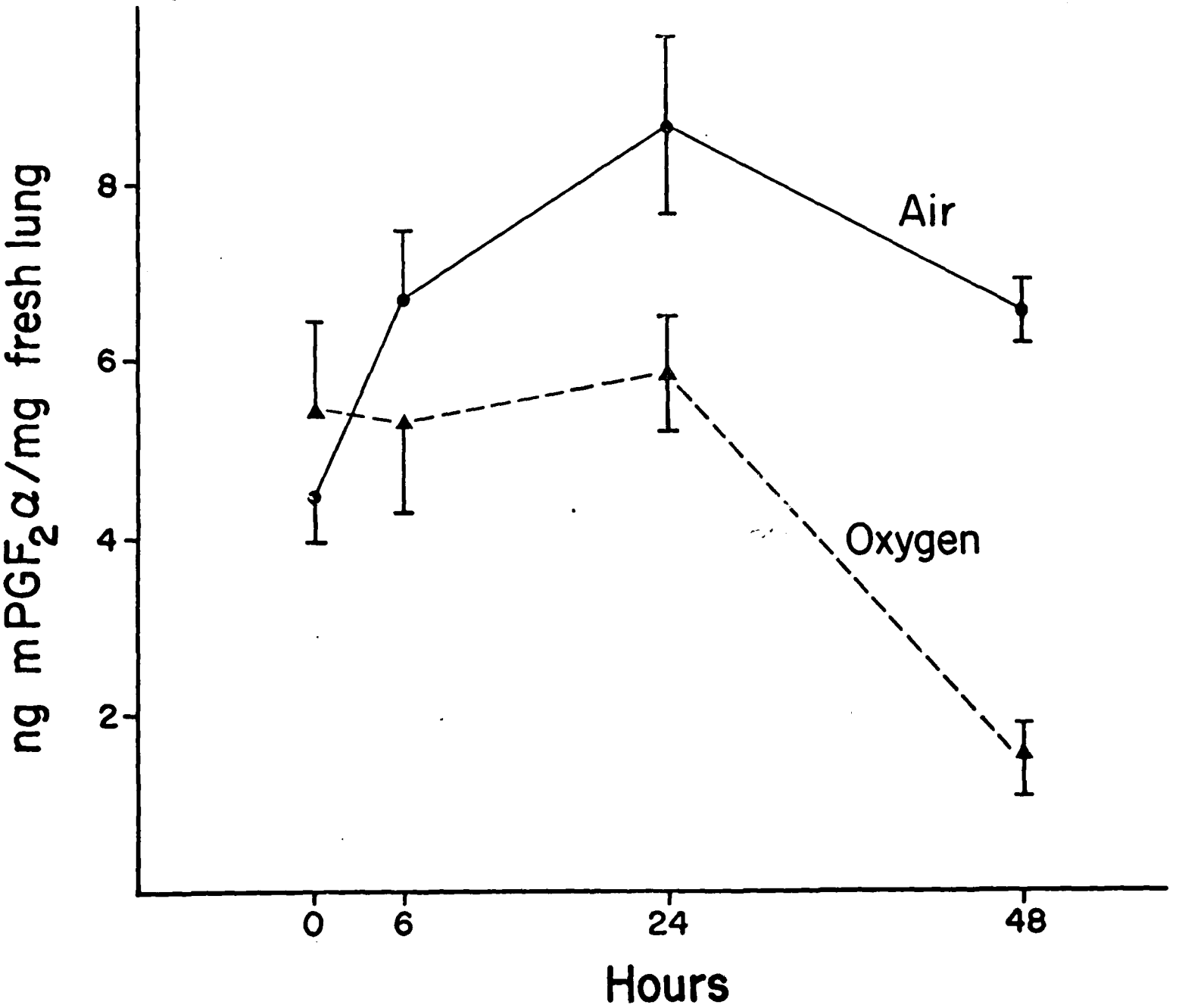
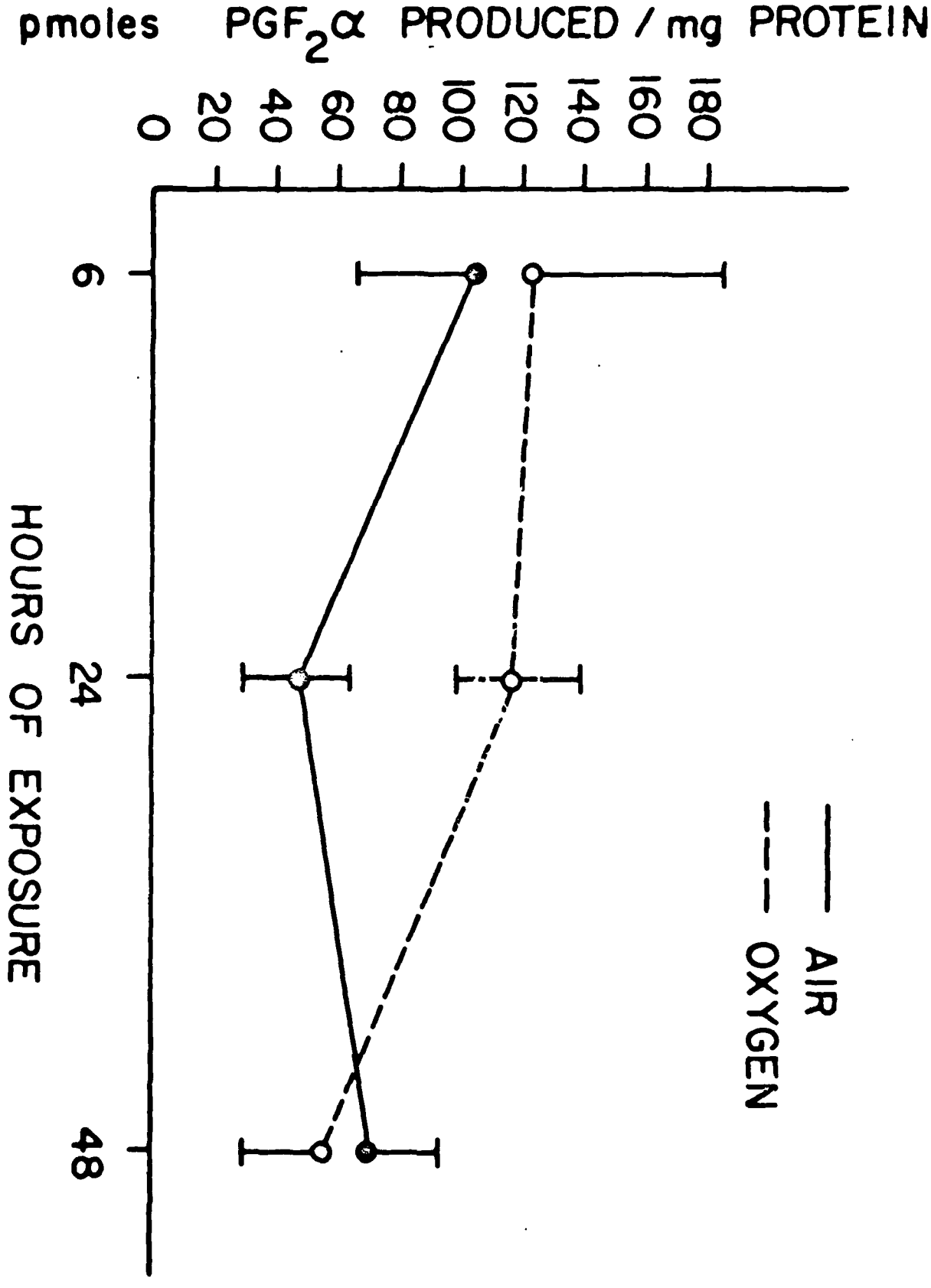

ng mPGF2α/mg fresh lung
8
6
4
2
Air
Oxygen
0
6
24
48
Hours


pmoles PGF2α PRODUCED / mg PROTEIN
0
20
40
60
80
100
120
140
160
180
6
24
48
HOURS OF EXPOSURE
AIR
OXYGEN

In the course of these experiments, we found the decrease of pulmonary prostaglandin dehydrogenase/reductase activity to be a sensitive indicator of biochemical toxicity, the most sensitive non-fatal parameter we have encountered. We assessed the effects of dietary fat content on relative susceptibility using it as a biochemical index of toxicity (Table 5)

% Dietary Fat	% Decrease in Enzyme Activity after 48 hours Hyperoxia (n = 8)
5.0	76
9.3	80
21.2	59
36.5	53

The general trend of a lesser decrease in enzyme activity with higher dietary fat content contrasted with increased mortality as a function of fat content (Table 4).

We used the same index to assess the efficacies of dietary supplementation with vitamin E and selenium (Tables 6 and 7).

Table 6: Percent reduction of enzyme activity following 48 hours exposure to hyperoxia (n = 8)

Dietary vitamin E (I.U.)	% Decrease
0	96
60	85
60 (injected i.p.)	89
600	52

Table 7: Percent reduction of enzyme activity following 48 hours exposure to hyperoxia (n = 8)

Dietary Selenium (ppb)	% Decrease
0	93
100	63
1000	88

These results suggest that supplementation with vitamin E at about 10 times the normal level can alter the symptoms of pulmonary toxicity, while supranormal supplementation with selenium does not.

MISCELLANY

The antioxidant enzyme glutathione peroxidase plays a role in prostoglandin synthesis and we wondered whether differences in its activity could change endogenous prostaglandin concentrations. The results in Table 8 suggest that its activity does not alter PG concentrations.

Table 8:

Tissue	Se (ppb)	GSH-Px (e.u./g)	Prostaglandins (ug/g/10 min.)			
			F_2	E_2	TxB_2*	6-keto-F_1 **
Lung	0	2	2.0	0.39	3.4	235
	100	20	2.2	0.42	3.1	325
	1000	23	1.7	0.30	3.2	223
Liver	0	15	1.2	0.42	0.08	0.28
	100	96	1.0	0.34	0.11	0.34
	1000	120	1.1	0.32	0.09	0.34

* Stable metabolite of thromboxand A_2
** Stable metabolite of PGI_2

We were also interested in the effects of aspirin on susceptibility to oxygen poisoning. It is commony believed that aspirin exacerbates the symptoms of toxicity but we are aware of only one report demonstrating experimental evidence, and that used unphysiologically high doses(3). If prostaglandins play a significant role in the etiology of oxygen toxicity, then synthesis-inhibition by aspirin should affect the onset of symptoms. The results of subcutaneously-administered aspirin on mortality in mice is shown in Table 9.

The results were equivocal. Controls at 1 ATA had lower mortality but about the same death times as those given aspirin. Mortality at 2 and 4 ATA was uniformly high for all groups. At 2 ATA, mean death time increased with dose at 4 ATA. These results did not permit a conclusion as to the effects of aspirin on development of oxygen poisoning.

Table 9: Percent mortality and mean ± S.D. time to death (minutes) of mice injected with aspirin prior to hyperoxic exposure (n = 12).

Aspirin, mg/kg	Oxygen, ATA 1	2	4
0	66% 6540 ± 840	100% 1356 ± 132	100% 128 ± 59
10	83% 6240 ± 780	90% 1344 ± 108	100% 151 ± 91
30	83% 6540 ± 1320	100% 1314 ± 60	100% 157 ± 76
50	83% 6780 ± 2760	90% 1296 ± 108	100% 213 ± 77

SUMMARY

This project has investigated two areas of dietary influence on susceptibility to oxygen poisoning about which conclusions can be drawn. First, supplementation with supranormal levels of vitamins, trace metals or amino acids which would seem to play a role in the antioxidant defenses of cells does not provide appreciable protection against toxicity except in the case of vitamin E. Although some of our evidence is conflicting, we conclude that dietary intakes of vitamin E of at least 10 times the daily requirement can impart some resistance to toxicity. The beneficial effect is small when compared to non-dietary techniques, such as pre-acclimization to hyperoxia, but is easy and apparently without untoward side effects. We can see no reason why supranormal dietary supplementation should not be used in persons who will undergo exposure to hyperoxia.

Second, there is some evidence that amount and possibly type of dietary fat will influence susceptibility to oxidant toxicity. How such an effect is mediated is not clear but synthesis and catabolism of prostaglandins is a good possibility. The lungs have one of the highest tissue concentrations of prostaglandins and is the major organ responsible for their clearance from the blood. The well-documented deterioration in the ability to detoxify prostaglandins with the onset of toxicity is a likely mechanism for some of the cardiovascular-related events in the etiology of oxygen poisoning. Because a role for prostaglandins in oxygen toxicity has not been demonstrated, we cannot advocate prophylactic steps which might be taken. If such a role is eventually proved, we have demonstrated that alteration of dietary fat content and composition can change tissue prostaglandin profiles. It might thus be feasible to optimize resistance to oxygen poisoning by changing prostaglandin metabolism via dietary treatment.

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PRESENTATIONS

In addition to the publications cited in this report, the following presentations were also made with support from this contract:

Susceptibility of altitude-acclimatized mice to acute oxygen toxicity, Annual Scientific Meeting, Undersea Medical Society, Washington, D.C., 1974.

Oxygen Poisoning Panel, Navy Biomedical Workshop, Panama City, Florida, 1976.

Dietary lipid and the possible role of prostaglandins in pulmonary oxygen poisoning, Annual Meeting, Undersea Medical Society, Toronto, Canada, 1977.

Dietary selenium and tocopheral as a possible prophylactic to pulmonary oxygen poisoning, VI International Conference on Hyperbaric Medicine, Aberdeen, Scotland, 1977.

Effect of dietary fat and aspirin on pulmonary oxygen toxicity, Annual FASEB Meeting, Chicago, 1977.

Response of rat lung prostaglandin metabolism to normobaric hyperoxia, Annual FASEB Meeting, Dallas, 1979.

Pulmonary prostaglandin metabolism during normobaric hyperoxia, VII Symposium on Underwater Physiology, Athens, Greece, 1980.

Dietary fat and oxygen poisoning, Nutrition Institute, Colorado State University, Fort Collins, Colorado, 1980.